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PB-285 923

First American-Soviet Symposium on the Biological Effects of Pollution on Marine Organisms

(U.S.) Environmental Research Lab, Gulf Breeze, FL

May 78

PB 285 923

United States
Environmental Protection
Agency

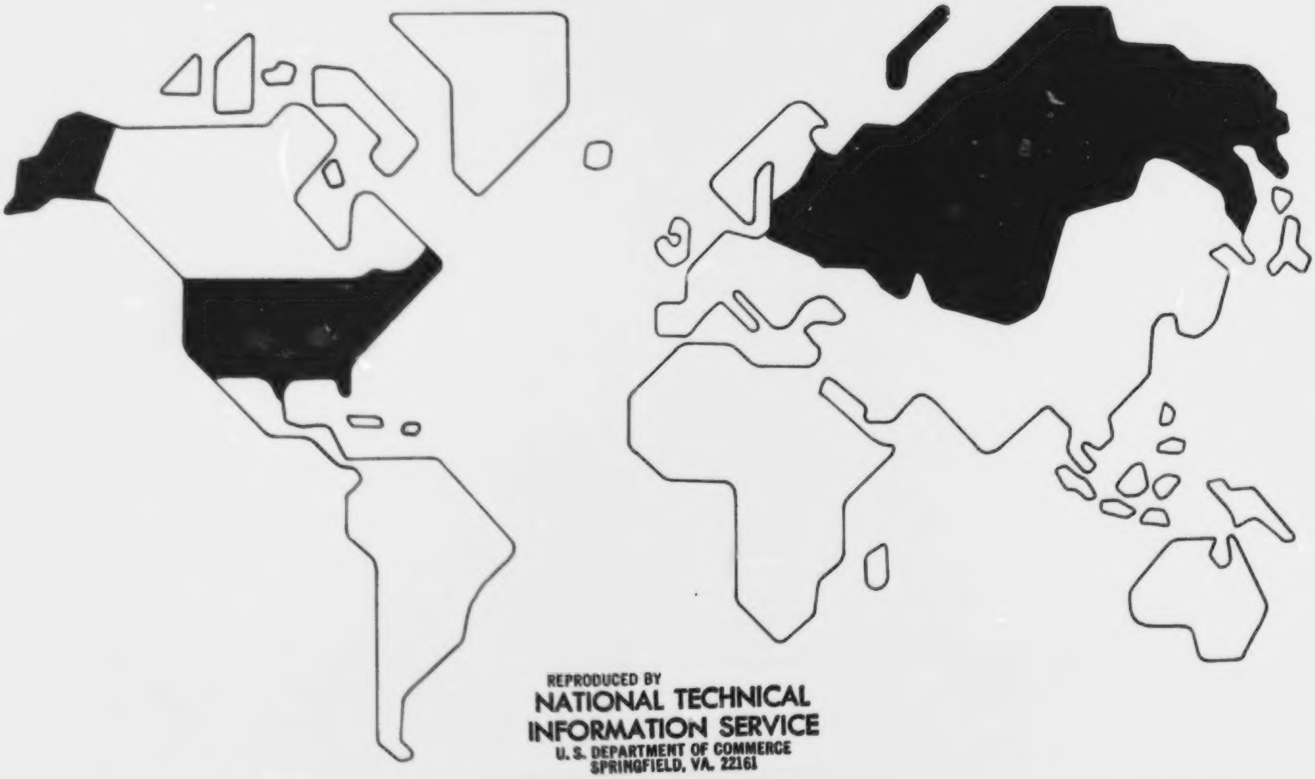
Environmental Research
Laboratory
Gulf Breeze FL 32561

EPA-600/9-78-007
May 1978

Research and Development

EPA

First American-Soviet Symposium on the Biological Effects of Pollution on Marine Organisms



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176

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/9-78-007

2.

3. RECIPIENT'S ACCESSION NO.
PB285923

4. TITLE AND SUBTITLE
FIRST AMERICAN-SOVIET SYMPOSIUM ON THE BIOLOGICAL EFFECTS OF POLLUTION ON MARINE ORGANISMS

5. REPORT DATE
May 1978

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, Florida

10. PROGRAM ELEMENT NO.
1BA608

11. CONTRACT/GRANT NO.
Joint Project VI-2.1

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Office of Research and Development
Washington, DC

13. TYPE OF REPORT AND PERIOD COVERED
Final, September 20-24, 1977

14. SPONSORING AGENCY CODE
EPA/600/4

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This symposium was conducted under a US-USSR Environmental Agreement, Project 02.06-21 titled "Influence of Pollutants on Marine Organisms." American and Soviet specialists discuss state-of-the-art for hydrobiological analysis of basic structural components of marine ecosystems and the influence of various pollutants on these components. Participants define problems related to methods for modeling the influence of pollutants on the marine environment, long-term forecasting and determination of permissible loads of pollutants, and the unification and intercalibration of methods for determining production of microorganisms of ocean bacterioplankton and phytoplankton. Results or laboratory research on the influence of pollution on the marine environment are presented. Proceedings were published in English and Russian in compliance with the Memorandum from the 4th Session of the Joint US-USSR Committee on Cooperation in the Field of Environmental Research.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Water pollution Marine organisms Modeling Oceanic ecosystems Photoplankton Oil Microcosms Radioactivity	Expotentially broken rod US-USSR Agreement on Cooperation in the Field of Environmental Protection Project 02.06-21	

18. DISTRIBUTION STATEMENT Unlimited	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 176
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE mF A01 PcA09

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/9-78-007

FIRST AMERICAN-SOVIET SYMPOSIUM
ON
THE BIOLOGICAL EFFECTS OF POLLUTION
ON MARINE ORGANISMS

SYMPOSIUM SPONSORED AS PART OF THE U.S.-U.S.S.R.
AGREEMENT ON PROTECTION OF THE ENVIRONMENT

by

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FOREWORD

Chemical pollution and the resulting impact on the World Ocean is particularly important among the numerous problems of environmental protection since the enormous area covered by the open waters of the World Ocean is not under the jurisdiction of any individual government. In many instances, man has turned the oceans which formerly separated countries and people into common reservoirs containing the discharges of wastes which accompany the production of materials. The accumulation and spread of pollution in the waters of the World Ocean can produce unforeseen and unwanted changes in the structure of functioning of natural communities. This, in turn, causes a large part of society to be concerned.

A similar situation is the incentive for coordinating the efforts of scientists from different countries to jointly develop questions for evaluating the quality of natural waters and the scientific basis for preventative measures to protect their living population.

The US-USSR Agreement on Cooperation in the Field of Environmental Protection, signed in May 1972, is an important step toward international scientific cooperation in solving these problems. One part of this agreement, Project 02.06-21, provides for a study of the "Influence of Pollutants on Marine Organisms." The creation of a program for global monitoring of pollution in the World Ocean is a necessary step for solving marine pollution problems. A system for observing the biotic component of the earth's surface water must hold an important place in such a system. Therefore, in the first stages, specialists should develop parameters characterizing the structural and functional properties of natural ecosystems, and likewise develop the possibility of studying them in laboratory conditions.

In this particular direction, joint Soviet-American studies under Project 02.06-21, "Influence of Pollutants on Marine Organisms," have developed within the framework of the US-USSR Environmental Agreement. The work performed laid the foundation for conducting the First Soviet-American Symposium on Hydrobiological Methods for Analyzing Marine Pollution, which took place September 20-24, 1976, at the Gulf Breeze (Florida) Environmental Research Laboratory. Soviet and American specialists presented more than 20 papers which gave a multiple exposure to the contemporary state of methods for hydrobiological analysis of basic structural components of marine ecosystems and the influence of various pollutants on them.

Problems which were discussed at the successfully conducted symposium (methods for modeling the influence of pollutants on the marine environment, long-term forecasting and determination of permissible loads of pollutants, unification and intercalibration of methods for determining production of microorganisms of ocean bacterioplankton and phytoplankton, methods for studying pollutants of varied nature and their influence on the environment in field and laboratory conditions, results of laboratory research on the influence of pollution on the marine environment) are undoubtedly of great interest to a large group of specialists who dedicate their activity to various scientific aspects of protecting the World Ocean from pollution.

Consequently, the co-chairpersons of Project 02.06-21 have agreed to publish the materials from the symposium, in Russian in the USSR and English in the United States, as stated in the Memorandum from the 4th Session of the Joint US-USSR Committee on Cooperation in the Field of Environmental Protection.

Thomas W Duke

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ABSTRACT

This symposium was conducted under a US-USSR Environmental Agreement, Project 02.06-21 titled "Influence of Pollutants on Marine Organisms." American and Soviet specialists discuss state-of-the-art for hydrobiological analysis of basic structural components of marine ecosystems and the influence of various pollutants on these components. Participants define problems related to methods for modeling the influence of pollutants on the marine environment, long-term forecasting and determination of permissible loads of pollutants, and the unification and intercalibration of methods for determining production of microorganisms of ocean bacterioplankton and phytoplankton. Results of laboratory research on the influence of pollution on the marine environment are presented. Proceedings were published in English and Russian in compliance with the Memorandum from the 4th Session of the Joint US-USSR Committee on Cooperation in the Field of Environmental Research.

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PROTOCOL

OF THE FIRST AMERICAN-SOVIET SYMPOSIUM ON THE BIOLOGICAL EFFECTS OF POLLUTION ON MARINE ORGANISMS

In accordance with the principles laid down in the Protocol from the Working Group Meeting on Project VI-2.1, held in the USSR in July 1976, and in the Memorandum of Implementation from the Fourth Meeting of the US-USSR Joint Committee on Environmental Protection, a joint US-USSR Symposium on the Biological Effects of Pollution was held in Gulf Breeze, Florida, September 20-24, 1976.

The Symposium was co-chaired by Dr. T. W. Duke, Director, Gulf Breeze Environmental Research Laboratory, and Dr. A. I. Simonov, Office Chief, State Oceanographic Institute Moscow. The support from the Gulf Breeze Laboratory was most helpful and services of interpreters were excellent. A list of participants is attached.

The participants presented prepared papers on biological effects of pollution on marine organisms from radioactivity, pesticides and metals and the design of methods of phytoplankton, and microbiological production, as well as the development and use of models to determine the movement of pollutants and biological impact of pollutants on the marine environment.

Stimulating discussions were held after each presentation and important aspects of these discussions will be included in the published proceedings of the Symposium. In addition, the following proposals were discussed concerning future joint research projects:

A. MODELING

Specialists discussed models now available, their strengths and weaknesses. It was proposed that this group jointly undertake the development and validation of models for some specific areas of the World Ocean. For example, a model for the calculation and prediction of primary production in the Gulf Stream including the system of the North Atlantic Current and models for microcosms were suggested.

B. PRIMARY PRODUCTION

Specialists having discussed existing methods for determining primary production came to the conclusion that there is a significant need for improving the methods--indeed, a need to create a new method. It was agreed that a joint effort to develop methods for determining primary productivity and effects of pollutants on primary productivity would be worthwhile.

C. MICROBIOLOGY

US and USSR specialists came to the conclusion that it is worthwhile to conduct joint microbiological studies in laboratory conditions and seawaters.

D. RADIOACTIVITY

Experts expressed an interest in obtaining more detailed information on the uptake--accumulation and effect of transuranic radionuclides on marine organisms. Also, they expressed the desire to conduct joint work on intercalibration of methods of analysis and with subsequent discussion at the next Symposium.

Both sides discussed and gave a high evaluation of the intercalibration results of hydrobiological methods of analysis made on the joint US-USSR cruise R/V *Moskovskiy Universityet* in July and August of 1975 and prepared a joint report for publication. The sides expressed desire that the American specialist, Dr. Iverson, a participant in the joint intercalibration, meet at Moscow State University (Moscow) in December of 1976 with his Soviet colleagues to complete the discussion of results on the intercalibration of hydrobiological analyses.

Both sides also agreed to publish the Symposium papers and highlights of the discussion during 1977.

The co-chairmen agreed to write the text of the Introduction to the Proceedings of the First Hydrobiological Symposium via correspondence by December 1, 1976. They also agreed to supply each side with final text of papers in both languages by April 1, 1977. Both sides agreed to publish the planned but not read papers as abstracts.

Members of this Symposium recommended that there be discussion of the results of the above mentioned studies and other joint studies at the Second Hydrobiological Symposium. It is desirable to hold this Symposium in the Soviet Union within the next one and one-half to two years.

Both sides expressed the desire to conduct expeditionary observations in 1977 or 1978 in the North Atlantic on the above mentioned questions. Co-chairmen will discuss the details of the expeditionary observations during 1977.

After the meetings, the Soviet scientists toured the research facilities at Florida State University in Tallahassee.

The Symposium and meetings were held in an atmosphere of friendly cooperation and have been of mutual benefit to both sides. The Symposium expressed its thanks to Dr. Duke, organizer of the joint meeting.

T.W. Duke

T. W. Duke

U.S. Leader of
Project VI-2.1

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DESIGN OF SIMULATION MODELS TO DETERMINE BIOLOGICAL IMPACT OF POLLUTANTS ON THE MARINE ENVIRONMENT

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INTRODUCTION

Models for simulating the behavior of ecological systems in their natural state or after an acute or chronic perturbation must be rigorous both in concept and in adherence to physical laws. The primary objective of this paper is to review the bases for constructing models incorporating systems' responses to pollution and to explore the conceptual bases for such models.

THE CONCEPTUAL BASES

POLLUTION DEFINED

Pollution may be defined as the inducement of a stress that can potentially drain energy from the normal functioning of the system. In response, affected organisms become less capable of performing their life-supporting functions and ultimately less competitive. The organisms are therefore subject to replacement by others better adapted to the prevailing circumstances. In severely polluted environments no organism may possess the necessary survival adaptations. By defining pollution in terms of energy, empirical measurement is facilitated and the effect of a pollutant is more easily incorporated in a multicomponent model.

POLLUTION AS A SOURCE OF POTENTIAL ENERGY

Essentially, the two main classes of pollutants are distinguishable on the basis of kind or type, and quantity or intensity. Some pollutants (for instance, atomic radiation, the heavy metals and certain organic biocides) provoke only negative responses from living organisms. The severity of response is generally proportional to the quantity or intensity of the pollutant. Other commonly cited pollutants (e.g., temperature, mineral nutrients, salinity, etc.) provoke negative responses only when present in quantities which the living organisms cannot tolerate. However, the same conditions may represent the optimum survival requirements for organisms in other systems where the same pollutant is present in natural quantity. This is consistent with the concept of the environmental continuum in which various species find optimum conditions as well as conditions under which they cannot exist. Thus, a stress and a drain of potential energy in one species may in fact be an auxiliary source of potential energy for another species (cf. Odum, 1971).

THE SPECIES VERSUS THE SYSTEM

A common inadequacy of many biological models, particularly those associated with a pollution problem, is the tendency to focus on parameters describing the component species and population assemblages, as opposed to parameters descriptive of the larger system. A species population may be completely lost from the system as a result of a pollutant. A population model may fail to indicate that a better-adapted species would be able to colonize the system to the point that its appropriate system function could be maintained. Likewise, a system's model emphasizing system parameters may faithfully simulate a stâble system function (such as community metabolism) without indicating that a particular component species of interest was lost. A model usually focuses on the problem at hand and the question(s) under study, while the empirical data must be obtained through a research design sensitive to both system and species parameters.

KINDS OF MODELS

Quinlan (1975) has determined that all models may be subsumed under one of three model categories, i.e., biodemographic, bioenergetic or biogeochemical. Each has specific attributes and disadvantages. Biodemographic models incorporate parameters descriptive of the dynamics of the species populations, but occasionally may indicate a population whose material content exceeds that available in the parent ecosystem. Bioenergetic models, like biogeochemical models, are conservative in that energy or matter may be fully accounted for in the model. Energy and mass balances in this context can be used to assure quality in a simulation exercise. Bioenergetic models, however, often assume that the participants in any process behave in proportion to their specific energy content. These models ignore, for instance, the synergistic effect of an interaction or the amplification effect of a feedback. Biogeochemical models provide the most complete representations. They are inherently conservative, and their processes are not allowed to continue if one or more reactants are depleted. Modeling methodology promulgated by Odum (1971) basically satisfied the principles of conservation and causality by assigning to model participants energy values proportionate to the quantity of the work the system can perform.

Many contemporary models, in addition to supporting research, propose to model energy and energy flow when, in fact, participants in the model are commonly stocks and flows of organic matter. (*Energy* is the capacity for doing work. The term *flow* in this context is a misnomer, for it associates a flow with a thermodynamic state.) To resolve this particular dilemma, one may either model carbon as a biogeochemical model or assign work-generating energy values of Odum's bioenergetic models.

ARE SIMULATION MODELS PREDICTIVE?

The prediction of a future state can be made only from a knowledge of past and present states and the experience gained by observing analogous, time-dependent situations. Once programmed, a model may be visualized as a hard-wired network of storages and flows. The pattern is not subject to change during simulation. If an altered real-world system results in a different pattern in the arrangement and linkage of components, this occurrence would not be observed during a simulation exercise. Thus erroneous conclusions could be inferred. One common method that partially allows for circuit changes during simulation is to program the alternative arrangements, using a logic program as a switching mechanism in some predetermined manner. This method, however, requires that the optional future states should be determined in advance, as well as the conditions for any given state. Obviously, one purpose of modeling is to gain an insight into future states and to suggest that answers used in formulating the questions are circular and contradictory. The preferred mode of model design sufficiently aggregates the participants in the model so that minor changes in the network pattern do not invalidate the simulation. After the behavior of the aggregated model is understood through simulation under varied conditions and is validated, the model then can be redesigned to include more detail about the participants. The art of knowing how to aggregate a biological model and how to select the level of necessary detail may transcend the importance of operational computer/mathematical techniques in a successful modeling exercise.

A MODELING LANGUAGE

The need to bridge the gap between real-world observations and the mathematical formulations of the operational models has evoked the use of symbolical languages that explicitly portray relationships (Forrester, 1961; Odum, 1971). These symbol languages permit stocks, flows, and various forms of interactions and processes to be visualized as a whole and subsequently to be parameterized for simulation exercises. The energy language of Odum (1971) is used here to review the construction of models that would convey the behavior of ecosystems and their forms of response to pollution.

DESIGN OF THE BIOLOGICAL MODEL

The simplest model that can be constructed to simulate the gross behavior of an ecological system aggregates the producers and the consumers as separate participants. It also links them with two flows representing the trophic relationship and the regenerative feedback of the elemental constituents of organic matter (Fig. 1). In this representation of the system, the sun, as the primary forcing function, powers the photosynthetic machinery that builds new organic materials out of the inorganic constituents. Both producers and consumers exhibit auto-catalytic properties that induce a growth response as some function of the available energy and matter. In the production-respiration model, two passive storages represent the system's total stocks of organic and inorganic matter. Because all real-world systems are open to flow of energy and matter, each passive storage has external inputs and outputs. This provision essentially couples the system of interest with the larger total system. Two heat sinks account for energy lost when work is done in accordance with the second law of thermodynamics, the bioenergetic mode. As models for biogeochemical cycling in a mass balance simulation, the heat sinks may not be necessary. Since this basic model is parameterized with empirical values and simulated, the basic patterns of productivity and respiration (i.e., community metabolism) can be evaluated in relation to the intensity of the driving force and the inputs and outputs of organic and inorganic materials. In its most complex form, this model is highly predictive but sacrifices detail.

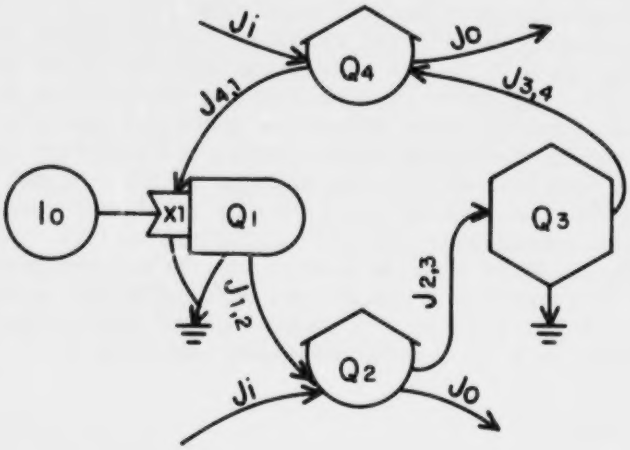

Figure 1. A basic conceptual model for an ecosystem emphasizing primary production and respiration with storages for organic and inorganic materials coupled to the larger system. Producers and consumers are auto-catalytic. Photosynthesis is the product of the interaction between sunlight and inorganic materials. Heat sinks account for the heat loss as a result of work. (Symbols from Odum, 1971)

I_o - sunlight

Q_1 - producers

Q_2 - organic materials

Q_3 - consumers

Q_4 - inorganic materials

J_i - inputs from source external to system of interest

J_o - output exported beyond system of interest

$J_{1,2}$ - net organic production

$J_{2,3}$ - consumption by consumers

$J_{3,4}$ - products of respiration

$J_{4,1}$ - uptake of inorganic materials

X_1 - photosynthesis (product of sunlight and limiting nutrient)

The basic model in Figure 1 can be progressively expanded by adding components to describe and detail specific functions associated with the system, provided the conservative characteristics are retained. In Figure 2, for example, gross primary productivity is modified by the circulation of water in such a manner that the uptake of the essential nutrients (and gases) becomes, over a certain range, a function of the delivery mechanism, irrespective of the total quantity available in the system. The interaction symbol is an expression of the limiting factor concept. Over the range in which one factor is limiting, the response is linear and operationally incorporated in the final simulation program as simple multiplication of the two interacting flows. (Whereas simple interactions are linear over a response range, the behavior of the system as a whole assumes nonlinear characteristics.) A second example of the expansion of the basic model is illustrated in Figure 3. Here, a stock of organic matter is detailed to include some component organic fractions of interest with respect to two distinct kinds of consumers. One consumer can draw nonselectively from either storage in proportion to its quantity. The second consumer is substrate specific. The total mass of consumers in this latter category varies with the quantity of material in this single storage.

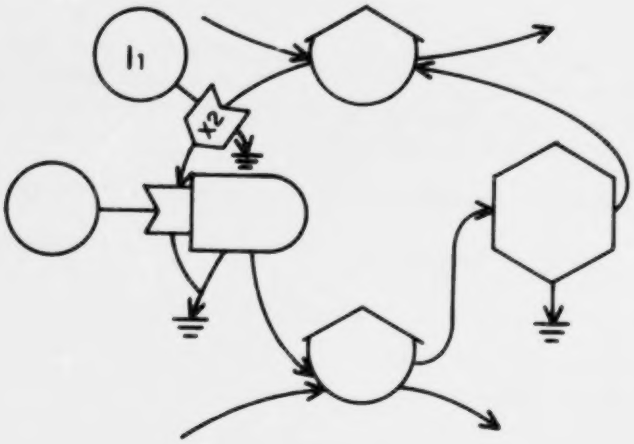

Figure 2. The basic production-respiration model illustrating the role of currents and circulation in juxtaposing inorganic materials and producers. (Symbols from Odum, 1971)

I_1 - circulation and currents

X_2 - stirring and transport of inorganic materials.

This mode of construction permits the resulting model to reflect the real-world system as seen through the eyes of the investigator. And, depending upon the question(s) being asked, no two models will be necessarily identical. In contrast, other modeling efforts utilize a fixed compartment-flow model in which the real-world system is superimposed by varying the size of stocks and by transferring coefficients linking them. Such models are generally preferred by investigators more interested in studying models per se than as tools in understanding the structure and function of a real-world system or specific problem.

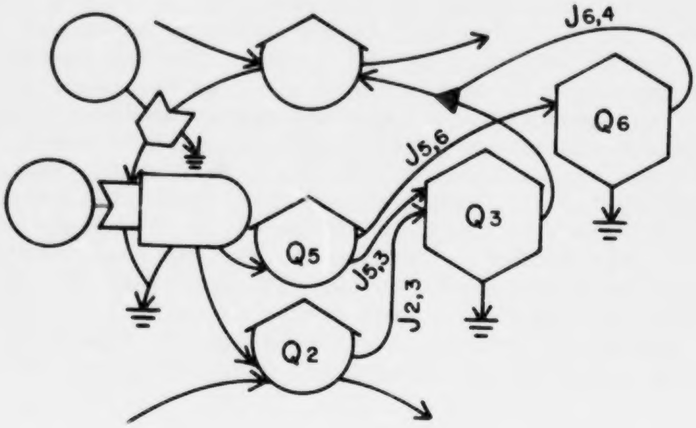

Figure 3. The basic production-respiration model with the storage of organic materials divided into two compartments based on composition and the relative feeding strategies of two groups of consumers. (Symbols from Odum, 1971)

Q_2 and Q_5 - organic matter of two different compositions

Q_3 and Q_6 - consumers with two different feeding strategies

$J_{5,6}$; $J_{5,3}$; and $J_{2,3}$ - consumption by respective consumer group

MODELING POLLUTANT IMPACT

The impact of pollutants on a biological system via its living components can be incorporated in a model in the same manner specific functions were included in the foregoing examples. Two examples of pollution-induced stress are provided in Figures 4 and 5, which respectively illustrate the species-selecting characteristics of temperature through the differential acceleration of metabolism and the variable response to an input of a biocide. Temperature (Fig. 4) is illustrated as having a similar overall response relative to each of the living components through the acceleration of metabolism such as might occur in the thermal plume of a coastal power plant. The two consumer participants in this example can be parameterized to show a differential response in the drain of metabolic energy for a given temperature. A similar acceleration of potential energy loss is shown for the producers, but in this instance temperature also is shown to have a positive amplification effect on primary production through enzymatic stimulation (Jorgensen and Nielsen, 1965). The result of this "push-pull" effect may be manifested during simulation as an increasing turnover time without changing the standing stock of producers (McKellar, 1975). Many other effects of elevated or otherwise altered temperature regimes can be similarly included in a model when specific relationships are known.

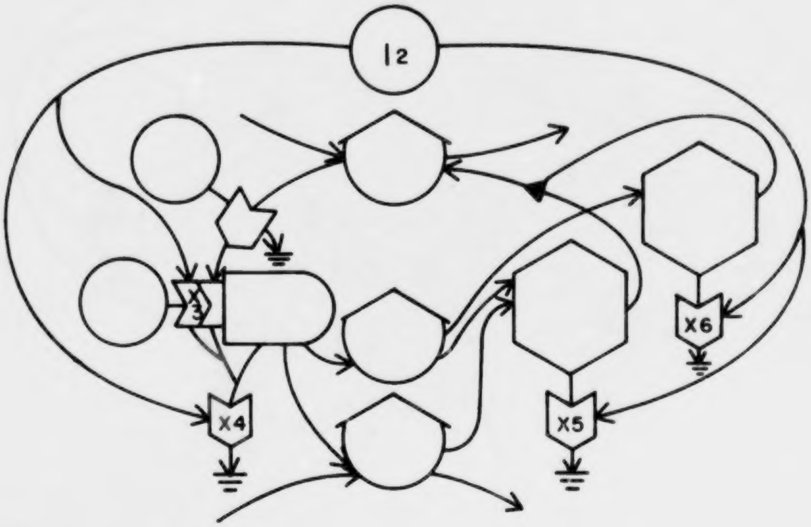

Figure 4. The impact of elevated temperature in accelerating the metabolism of living organisms illustrated as drains of potential energy. Differential temperature responses by the component species would be represented by the appropriate coefficients on the energy drains. Producers may be stimulated by small temperature rises offsetting the accelerated metabolic losses (Symbols from Odum, 1971)

I_2 - source of heat raising ambient water temperatures

X_3 - temperature stimulation of gross primary production

X_4, X_5 and X_6 - temperature-induced acceleration of metabolism

Provisions are included in the model in Figure 5 for the input and effect of biocide, which for discussion here is illustrated as a lipid-soluble chlorinated hydrocarbon. It is diagrammed as being introduced into the pool of materials available for uptake by the consumers. However, this kind of compound is basically insoluble in water and is absorbed onto the surfaces of solid substrates. Now consider the two stocks of organic material as contrasting-sized classes of organic particulates. Because of surface-area-to-volume ratio differences, the two-particle classes differentially sorb the biocide. The result is that one fraction carries a more concentrated biocide burden, thus affecting the two consumer groups in different degree, depending on their time-varying feeding strategies. The drain of potential energy on the consuming organisms can be incorporated in a simulation model as a metabolic acceleration (similar to that diagrammed for temperature), with the rate of potential energy loss proportional to the body burden.

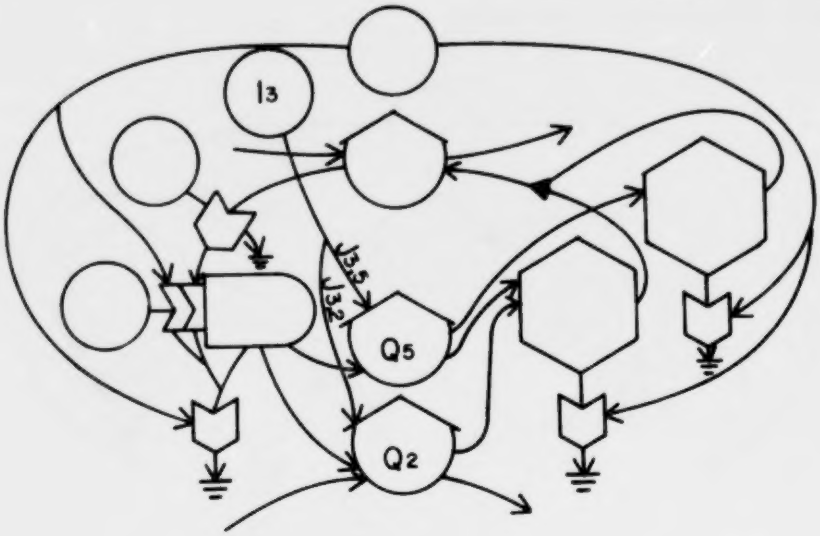

Figure 5. The introduction of a chlorinated hydrocarbon entering two organic storages differing in surface-area-to-volume ratios. The water-insoluble biocide sorbed onto the surfaces of the organic materials would result in different concentrations and consequently different doses to the consumer based on their time-dependent feeding strategies. (Symbols from Odum, 1971)

I_3 - source of chlorinated hydrocarbon

Q_2 and Q_5 - organic particulates of two different size classes

$J_{3,5}$ and $J_{3,2}$ - differential rates of absorption onto particulate surfaces

The model in Figure 5 represents a summation of Figure 1 plus the addition of four components to include specific circumstances. Other components could be added in like manner for greater detail or for incorporating other specific circumstances. At any level of increasing complexity, given the pertinent data, simulations could be made for such purposes as understanding the behavior of systems with these characteristics or for developing hypotheses for subsequent testing.

SUMMARY

Simulation models to determine the biological impact of pollutants can assume any one of three forms (biodemographic, bioenergetic or biogeochemical) if they are conservative and mimic real-world, cause-effect processes. The term *pollution* is sometimes difficult to define precisely. It can be modeled relatively easily, however, when one considers a pollutant as an inducement of a stress that alters the potential energy allocation process of a system. (More energy goes into stress-compensating processes and less into competition.) Depending on the kind and quantity of a pollutant, the impacted system may exhibit many different types of compensatory strategies. The possibility that a pollution-induced response may be manifested in a significant alteration of the basic network pattern of the system argues for highly aggregated models that are insensitive to internal network changes. The modeling methodology of Odum (1971) is particularly useful in the design of simulation models to determine the biological impact of pollutants, because rigorous models can easily be constructed to include any observable and measurable process. Simulation exercises are viewed here as a tool for understanding system processes, rather than as the end result of a research task.

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PROBLEMS IN MODELING THE EFFECT OF POLLUTION ON BIOLOGICAL SYSTEMS OF THE OCEAN

by

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The increasing tempo of industrialization, the intensification of agriculture, the rapid growth of population have given rise to rapidly progressing pollution of the biosphere, the extent and rate of self-purification of which are limited. To prevent an ecological crisis, special investigations are necessary both in the control of the processes of pollution of ecosystems as well as in the change in their functioning under conditions of pollution.

MODELING OCEANIC ECOSYSTEMS

From the point of view of anthropogenic pollution of the ocean, it is significant that it first affects the surface layers, i.e., the most productive ecosystems of euphotic waters, which are the energy provision for the existence of the entire population of the ocean. It is natural that the investigation of the influence of pollution on the surface associations is of greatest importance.

Up to the present, extensive materials have already been collected about the biology of marine organisms, their interrelations and relations with the abiotic medium, including anthropogenic effects. There has arisen the practical possibility of beginning to analyze the functioning of associations under various external conditions. The complexity and variability of ecosystems and the difficulties of investigations arising in connection with this have forced particular attention to be directed to modeling the processes taking place in them. It makes it possible to describe the set of interactions in the ecosystem and not only to predict with sufficient validity the behavior of the system with a change in one or another of its parameters or one or another of the external effects, but also to evaluate certain situations arising in real systems and amenable with difficulty to direct measurement.

Works in producing mathematical models of various processes occurring in marine, primarily pelagic, ecosystems and models of the general pattern of the functioning of these ecosystems have been conducted during recent years in the Soviet Union. Some of the results obtained indicate that the models are quite adequate (Vinogradov et al., 1972, 1973). This has made it possible to carry out computer experiments with these models and, in particular, to evaluate the effect of the various factors of anthropogenic pollution on the functioning of natural systems (Vinogradov et al., 1975).

Another approach which at present is still only beginning to be developed, is the production of special models of the influence of pollutants on ecosystems, where the pollution is considered as some stochastic process (Brusilovskiy, 1975).

I will discuss below some of the results and proposals resulting from the works of collaborators of our laboratory and my colleagues, primarily B. S. Fleyshman, P. M. Brusilovskiy, O. G. Mironov, E. A. Shushkina, V. F. Krapivin and V. V. Menshutkin.

DETERMINISTIC MODELS OF PELAGIC ECOSYSTEMS

Investigations must, of course, begin with the simplest and most accessible for studying systems. Pelagic ecosystems are such systems. The significant homogeneity of the biotope causes the decisive situation in pelagic associations of having trophic relations, which are comparatively easy to evaluate quantitatively. The abiotic conditions, including the effect of pollutants, which have a direct influence on the functioning of pelagic associations can be determined and quantitatively evaluated comparatively easily. At later stages, when methods for investigating pelagic associations are available, one will be able to turn to the study and prediction of the behavior of systems including the population of both the water strata and the bottom of the sea.

The structure of models must be based on dynamic representations of the succession of pelagic associations (Vinogradov, Gitelzon and Sorokin, 1970), i.e., with the development of the association with time, its structural and functional properties vary and consequently their response to external unfavorable effects.

In considering pelagic systems of the open ocean, it is necessary to take into account that the system developing in time together with the current of "aging" water moves in space. Thus, one must consider as the elementary unit being modeled the succession of the association on the entire path of water from the time of ascent into the euphotic layer to the time of descent. In other words, one must model the temporal change of an association moving in space. Of course, there cannot be equilibrium (energy balance) of the system at each moment of time. The accumulation of energy takes place in the system close to the zone of formation, while its dissipation occurs "downstream." It is in the "accumulation period that the system reacts particularly strongly to inhibiting factors" (Federov, 1975). Thus, pollution is most harmful in the zones of system formation--upwellings and divergences of flow in the tropics or during the spring development of plankton in temperate latitudes.

According to the approach developed by Lyapunov (1963) and Odum (1972), the ecosystem can be represented in the form of a set of individual elements functioning relatively autonomously, between which are communications channels. The role of signals passing along these channels can be performed by certain portions of matter (energy) or information. One can accordingly distinguish the material and information communications between the elements. One can also distinguish two methods of transmitting matter and energy from one element of the ecological system to another. The first (flow) is necessarily related to some transformation of matter or energy. The second (transfer) is related only to active or passive displacements of the elements in the water stratum. The entire ecological system can be divided into cells such that within each cell the elements communicate only by flow, while transmission of matter and energy between cells is accomplished by transfer. The cell of a pelagic ecosystem of the tropic ocean is presented in Figure 1 as an example. It is evident from Figure 1, where the effect of pollution was not evaluated, that such effects can be taken into account.

However, it is obvious that to construct an adequate model it is necessary to have a sufficiently complete representation about how matter and energy are propagated between the corresponding elements, what regularities and influences determine the intensity of flow between the elements, what enters the system, what leaves or is extracted from it and in what amount. In particular, it is necessary above all to investigate experimentally the directionality and degree of effect of various pollutants on the basic living elements of the system. Without such estimates, any model cannot rise from the level of purely qualitative speculations and loses its predictive properties.

In having a representation of the flow of matter within a cell and the regularities of its transfer from cell to cell, one can describe the functioning of an ecosystem (association) with a system of balance equations, which have the form:

$$\frac{dT}{dz} = -aT$$

$$\frac{\partial n}{\partial t} = -h\,P_p + Dd + \eta \sum_{i=p,\,b,\,f_1 \ldots\ldots f_4,\, s_1 \ldots s_3} R_i + k\,\frac{\partial^2 n}{\partial z^2} + \beta\,\frac{\partial n}{\partial z}$$

$$\frac{\partial p}{\partial t} = d\,P_p - R_p - \mu p - \sum_{j=f_1 \ldots f_4} C_{pj} + k\,\frac{\partial^2 b}{\partial z^2} - \omega_1\,\frac{\partial p}{\partial z}$$

$$\frac{\partial b}{\partial t} = P_b - R_b \quad \mu b - \sum_{j=f_1 \ldots f_4} Cb_j + k\,\frac{\partial^2 b}{\partial z^2} - \omega_2\,\frac{\partial b}{\partial z}$$

$$\frac{\partial X_i}{\partial t} = u \sum_{i=p,b,d,f_1 \ldots f_4,\, s_1 \ldots s_3} D_{ji} - R_i - \mu_i X_i \quad - \quad \sum_{j=f_1 \ldots f_4,\, s_1 \ldots s_3} C_{ij}$$

$$\frac{\partial d}{\partial t} = \sum_{i=f_1 \ldots f_4,\, s_1 \ldots s_3} (H_i + \mu_i X_i) \quad - \quad \sum_{i=f_1 \ldots f_4} Cd_i + k\,\frac{\partial^2 d}{\partial z^2} - \omega_3\,\frac{\partial d}{\partial z}$$

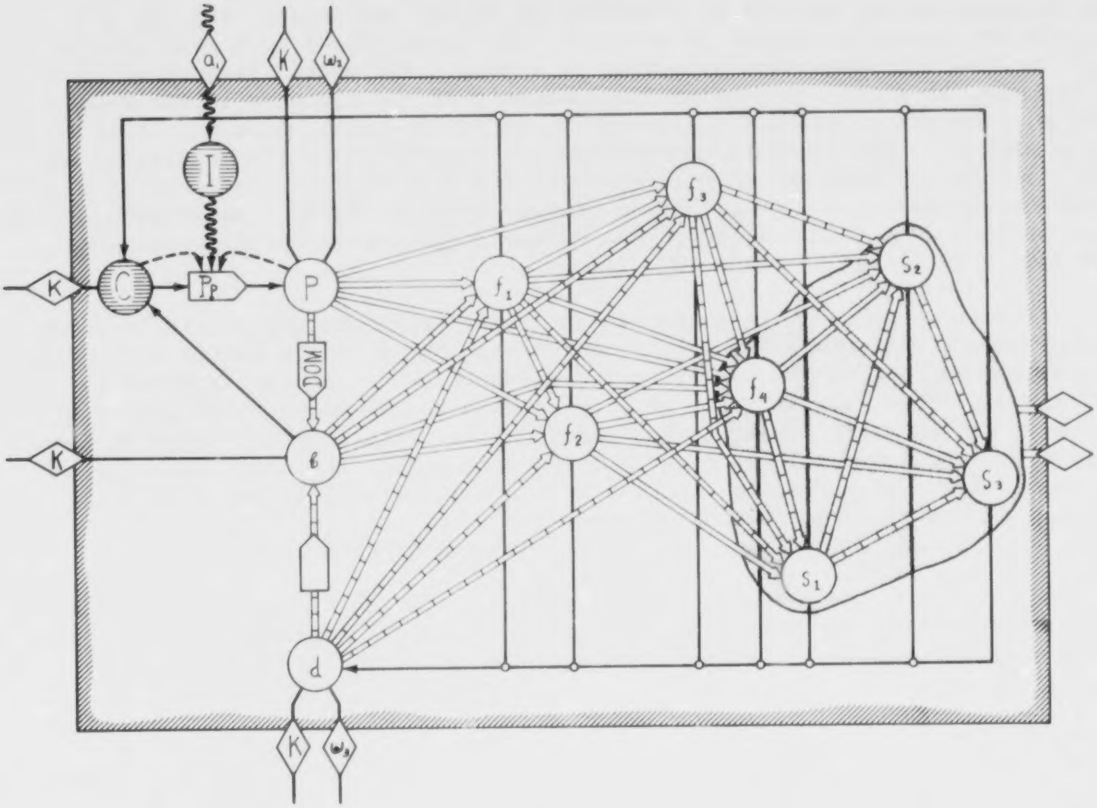

Figure 1. Block diagram of cell in the model of the functioning of a pelagic ecosystem of the tropical regions of the ocean. 1--living elements of the ecosystem; 2--inert elements of the ecosystem; 3--groups of elements; 4--flow of matter; 5--trophic communications; 6--transfer of matter; 7--solar radiation energy; 8--information communications; 9--transfer between cells; 10--ecosystem cell. k--turbulent diffusion; ω--gravitational precipitation of phytoplankton and detritus; T--solar radiation; C--biogenic elements; d--detritus concentration; DOM--dissolved organic material; p and P_p--biomass and production of phytoplankton; q--biomass of bacteria; f_1--biomass of protozoa; f_2--biomass of microzooplankton; f_3--biomass of small plant-eating animals; f_4--biomass of large plant-eating animals; s_1--biomass of cyclopoida; s_2--biomass of predatory copepoda; s_3--biomass of other predators.

where n is the concentration of biogenic elements; p and b are the biomass of phytoplankton and bacteria, respectively; f_1-f_4 are the various groups of plant-eating zooplankton; s_1-s_3 are various groups of predatory zooplankton; d is the concentration of detritus; t is the time; z is the depth; T is the light intensity; a is the light absorption coefficient; h is the biogen consumption coefficient in photosynthesis; P_p is the primary production*; D is the coefficient of biogen evolution as a result of detritus (d) decomposition; η is the evolution coefficient of biogenic elements in the metabolism process; R_i is the nutritive ration of the i-th element; k is the vertical diffusion coefficient; μ is the natural mortality coefficient; ω_1 and ω_3 are the rate of gravitational precipitation of phytoplankton and detritus; X_i is the biomass of the i-th element of zooplankton; C_{ij} is the partial ration of the i-th food consumer due to the j-th food supply; H_i is the unassimilated food of the i-th element of zooplankton.

It is obvious that terms taking pollution into account can be introduced into all the equations for phytoplankton and zooplankton. However, the corresponding coefficients must be experimentally evaluated for computer checking of the model.

The proposed model makes it possible to predict the behavior of the system with time: the change in biomass of the evolved elements with the development of the system (Figure 2) and the change in its vertical distribution. Comparison of the calculations made according to this model with the actual pattern observed in the ocean gives acceptable agreement (Vinogradov et al., 1973), which permitted us to use it for experimental purposes, explaining how a change in one or another of the parameters and communications affects the development of the system (Vinogradov et al., 1975). With the introduction of the effect of pollution, one can obviously predict its influence on the developing system.

It is likely that models of the development of associations over the reservoir area must have great value in evaluating the effect of pollutants. One can easily convert to such models from that just considered by introducing transfer of the elements by currents into the scheme. We performed such an experiment with the example of the Sea of Japan (Menshutkin, Vinogradov and Shushkina, 1974). The cell presented in Figure 3 was taken and the surface flow diagram of Figure 4, by which the transfer of plankton was accomplished. The motion of nekton (calmar and fish) was assumed active. After assuming certain other initial and boundary conditions, we successfully obtained the pattern of the quantitative distribution of the studied elements over the entire water area of the sea and its annual dynamics (in increments of five days) (Figure 5).

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* $P_p = P_{max} \frac{T}{T_{opt}} \exp\left(1 - \frac{T}{T_{opt}}\right)(1 - 10^{-0.1\,n})^{0.6}$

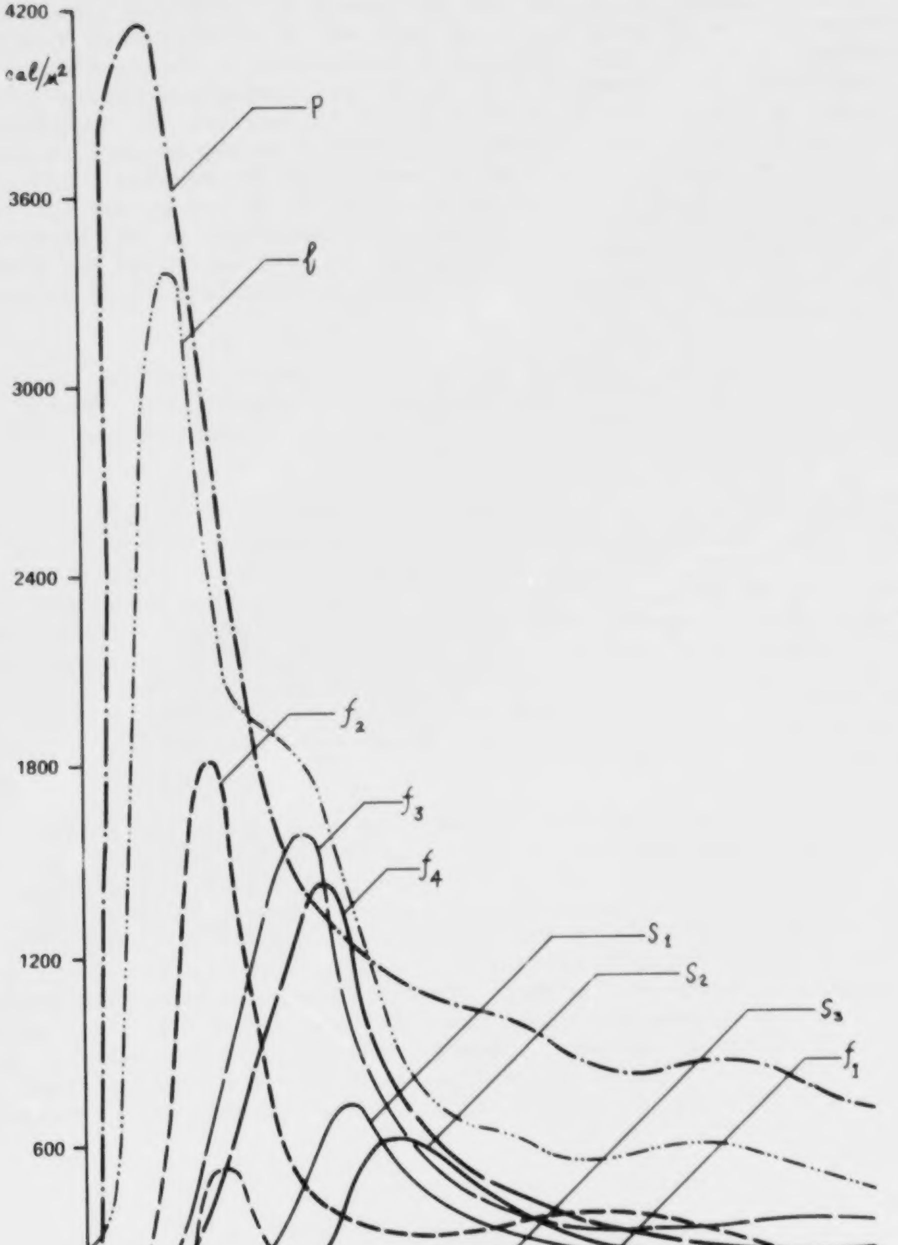

Figure 2. Modeled changes in biomass of the various elements of the association of the tropical regions of the ocean with their development. Notation as in Figure 1.

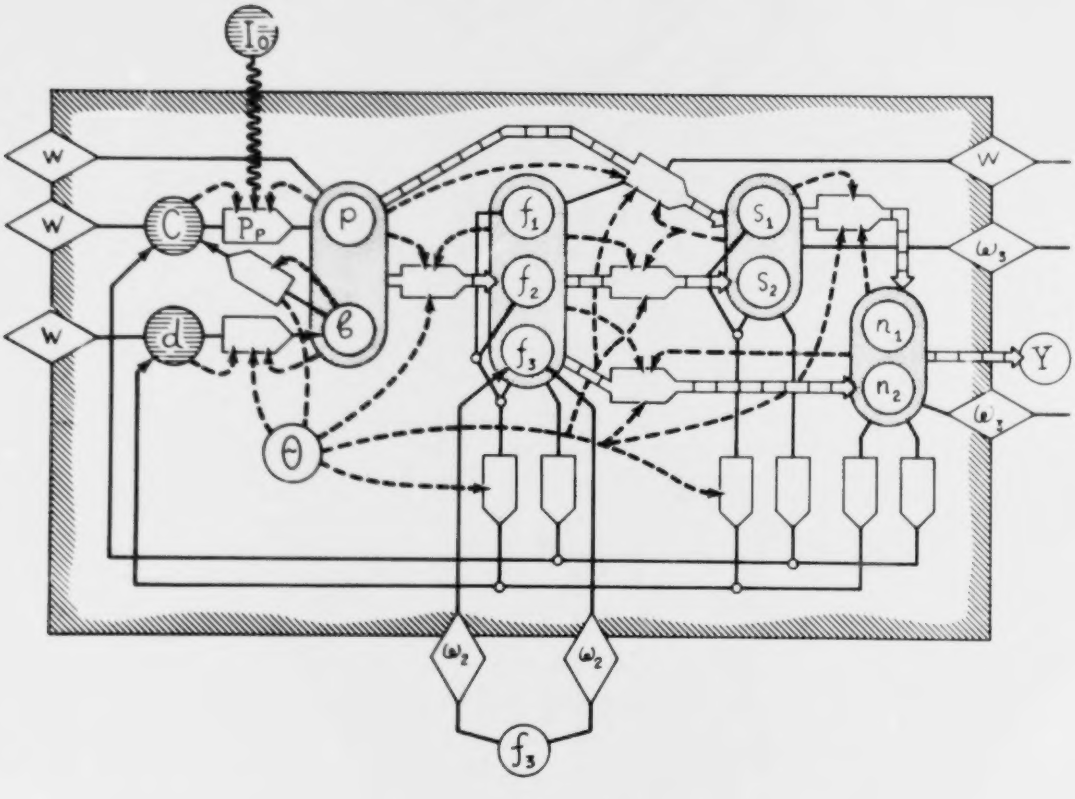

Figure 3. Block diagram of cell in the model of the functioning of the pelagic ecosystem of the Sea of Japan: f_1--biomass of boreal plant-eating epizooplankton; f_2--biomass of warm-water plant-eating epizooplankton; f_3--biomass of interzonal plant-eating zooplankton; s_1--biomass of the eurifag and predators of the boreal complex; s_2--biomass of the eurifag and predators of the warm-water complex; n_1--biomass of fish; n_2--biomass of calmar; θ--water temperature; w--current transport; Y--effect of fishing; ω_2--seasonal vertical migrations of interzonal plankton; ω_3--active migrations of fish and cephalopods. Remaining notation as in Figure 1.

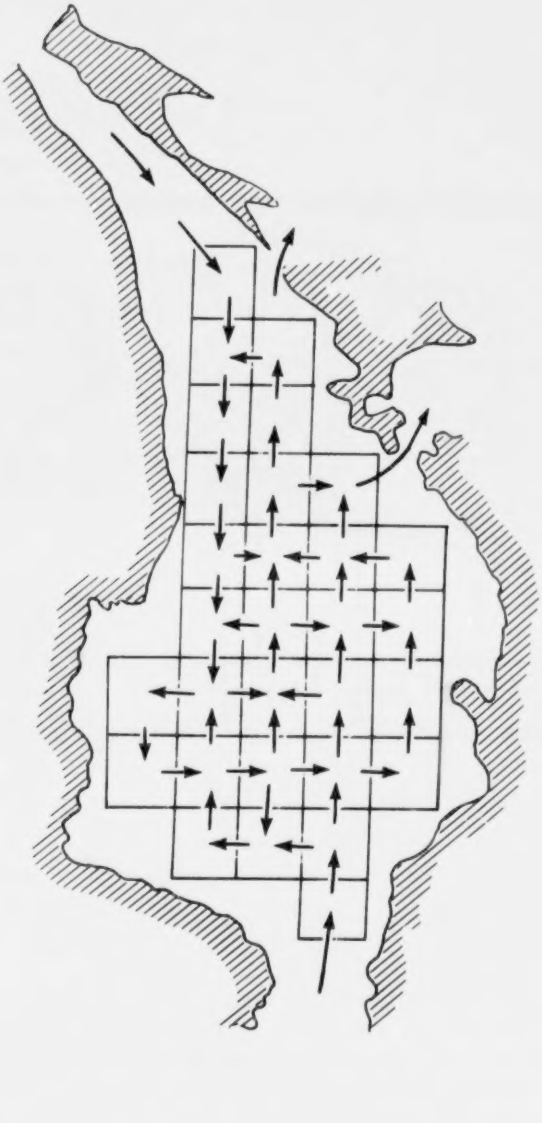

Figure 4. Diagram of the distribution of ecosystem cells and the direction of currents in the Sea of Japan.

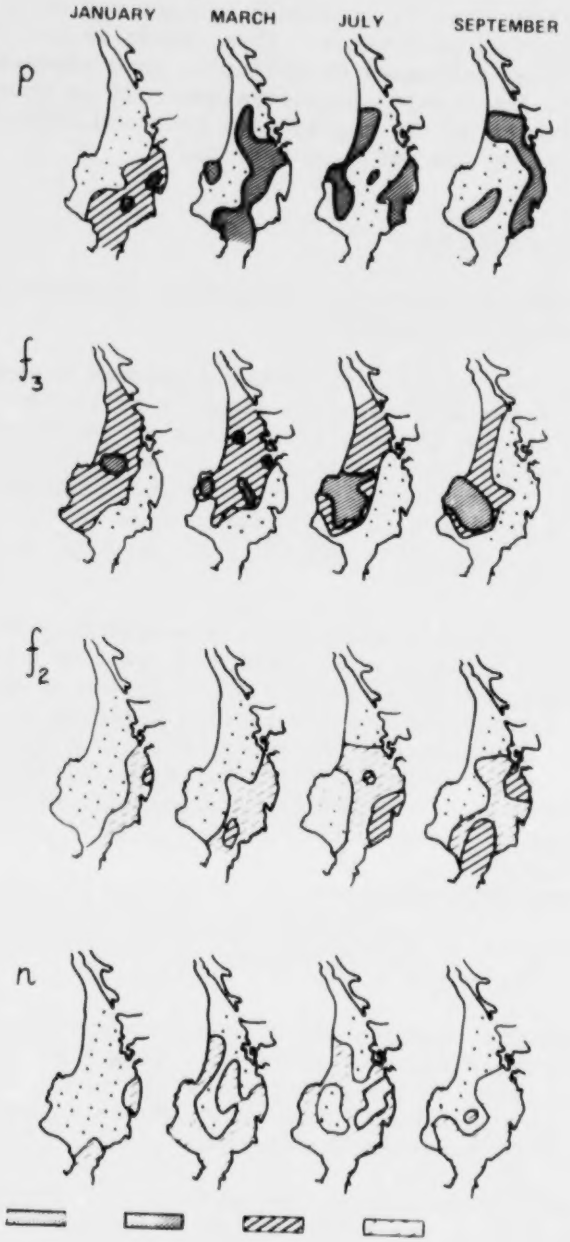

Figure 5. Modeled results of the distribution of certain elements of the ecosystem over the water area of the Sea of Japan (Menshutkin, Vinogradov and Shushkina, 1974): p--phytoplankton; f_2--warm-water plant-eating zooplankton; f_3--interzonal plant-eating zooplankton; n--nekton (fish and calmar); 1--biomass greater than 10 kcal/m^2; 2--10-5 kcal/m^2; 3--5-1 kcal/m^2; 4--1-0.1 kcal/m^2. Points denote cell centers.

Again, it is obvious that an analogous pattern can be obtained with consideration of the effect of pollutants. Thus, there is now the practical possibility of including the influence of pollution into adequate deterministic models describing the functioning of biosystems. It is necessary for this to obtain practical estimates of the suppression or stimulation of the activity of mass hydrobionts by the basic pollutants.

THE STOCHASTIC NATURE OF POLLUTION

Let us now consider approaches to evaluating the penetration of pollutants into the system and its response.

The incidence of the pollutant into the ecosystem occurs at random moments of time and in random amounts. The sequence of incidence of the pollutant is a sequence of similar events, which begin at random intervals of time, i.e., they are a random flow. This idea is the basis of stochastic models of the pollution of ecosystems. Another random quantity X_i--the intensity of the incidence--corresponds to each random value of t_i--the time of the i-th incidence of the pollutant. The set of pairs (t_i, X_i) is the pollution flow.

The pollution flow gives a comprehensive characteristic of the situation, but does not carry any information about the reaction of the system to the pollution. This reaction is composed of a biotic and an abiotic component. For example, an oil film on the sea is dissipated as a result of the evaporation of the oil, drifting into the atmosphere along with spray from waves, coagulating and settling, but also as the result of the consumption by various oil-oxidizing microorganisms and the accumulation of the oil hydrocarbons in the body of other hydrobionts.

In this case, the pollution level is determined by the expression:

$$\eta(t) = \sum_{t_i \leq t} f_i(t - t_i, X_i)$$

One can consider the reaction of an ecosystem not only to one but to several pollutants with an additive or synergetic effect (Brusilovskiy, 1975). Thus, the macroscopic pattern of the pollution of the ecosystem and the evolution of the pollution are described. These problems go beyond the scope of this report, and I will not consider them in detail.

In the first part of the report, we spoke of the possibility and necessity of taking into account the effect of pollution on the functioning of an ecosystem, about the microscopic pattern of pollution. The method of this accounting can be demonstrated with an example of the clearly simplified model of the Ferchulst-Pirl population:

$$\frac{dx}{dt} = x[E(\theta) - \gamma(\theta)x],$$

where x(t) is the population at time t^{θ}, (t) is some parameter symbolizing the state of the medium at time t, $E(\theta)$ is the natural (inherent) rate of increase of the population, $\gamma(\theta)$ is the coefficient of self-suppression of the population growth. If the pollution process forms a pollution level of the ecosystem $\eta(t)$, then the model of the population, the component of the ecosystem subjected to pollution, can be described as:

$$\eta(t) = \sum_{t_i \leq t} f(t-t_i, X_i)$$

$$\frac{dx}{dt} = X \cdot E[\eta(t)] - X^2\gamma[\eta(t)]$$

The change in the population will not be a deterministic function, but will be determined by the realization of the pollution level, i.e., the random process.

THE PROBLEM OF THE CONTROL OF POLLUTION PROCESSES

However, the model of the functioning of an ecosystem under pollution conditions is itself only a particular case of a more general model taking into account the presence of control. Pollution can then be taken in the broadest sense as any anthropogenic influence, and control in the sense of the sound optimum use of nature.

The problem in such a general formulation already goes beyond the scope of individual natural sciences and becomes the subject of engineering ecology. It uses analytic, not graphical, optimization models of theoretical cybernetics (systematics) with the apparatus of information and control theory. The basic premise of these investigations is that the probability characteristics of the pollution level of an ecosystem depend on the amount of resources available for fighting the pollution. The problem, as Brusilovskiy notes, reduces to how the available expectation of the pollution level is the minimum possible constant number for a definite period of time, and the dispersion of the pollution level is less than a definite specified value. The inverse problem is also of interest.

As a result of solving the direct problem of the distribution of resources, it appears possible to "freeze" the growth of the pollution level of the ecosystem at the minimum possible value for a given cost, while as a result of solving the inverse problem--to determine what amount of resources (cost) is necessary in order that this "frozen" value be then specified.

CONCLUSION

The presented reports were to serve as illustrations of the obvious statement that the problem of evaluating the effect of anthropogenic pollution on the biological systems of the ocean should now be approached from two directions:

1. to obtain concrete data about the nature and degree of the effect of the most widely distributed pollutants on mass forms (or groups) of hydrobionts, which will permit taking this effect into account in the available

"graphical" models of ecosystems and predicting their development.

2. to develop mathematical models taking into account the stochastic nature of pollution and the possibility of controlling its extent. Some fundamental aspects of the development of this direction are discussed in the report of V. D. Federov.

Note: The illustrations to this report are taken from: Vinogradov, M. E., and V. V. Menshutkin. 1976. The Modelling of Open Sea Ecosystems. Vol. 6, The Sea.

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AMERICAN METHODS FOR MEASURING PHYTOPLANKTON PRODUCTION IN THE OPEN OCEAN[1]

by

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INTRODUCTION

This paper summarizes the basic methodology for measuring primary production in the open ocean. There are other excellent publications which describe production measurement, most notably that of Strickland and Parsons (1972). Our publication supplements these already existing manuals and makes note of most recently discovered inadequacies of the ^{14}C technique. Problems include those of ^{14}C isotope quality, high dark-bottle counts, scintillation fluors, loss of ^{14}C when drying filters, etc. We also present, where possible, ways of overcoming these problems on the basis of our experience and that of others. The methods described here are designed for primary production measurement in the open ocean where there is a low concentration of particulate matter, high light penetration, and a relatively constant concentration of dissolved inorganic carbon. With modification, as noted by Strickland and Parsons (1972), the methodology presented here can be adapted to use in relatively low salinity water of high detrital content.

METHODOLOGY

A. SAMPLING DEPTHS AND LIGHT TRANSMISSION IN THE EUPHOTIC ZONE

Samples for production measurements should be collected from at least five depths based on irradiance values relative to that at the sea surface. The exact irradiance levels are chosen to agree with the transmittance of neutral-density screens used for shipboard incubation, but should be approximately 100, 50, 25, 10, and 3-1% of surface (0 meters) irradiance. Strong chlorophyll maxima should also be sampled.

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[1] Supported by NSF Grant DES76-01405. Contribution No. 146 from the Marine Sciences Research Center, State University of New York at Stony Brook.

Irradiance values should be measured with a cosine-corrected detector which is equally sensitive to either energy flux (μwatts cm^{-2}) or quantum flux (einsteins $cm^{-2}sec^{-1}$) over the entire photosynthetically active region of the spectrum (350-700 nm). The LAMBDA Instruments Corp. (Lincoln, Nebraska 68504) markets a suitable underwater quantum sensor and meter that is calibrated in terms of μeinsteins $m^{-2}sec^{-1}$. Regardless, the manufacturer's type and the spectral response of the underwater detector should be clearly stated.

We recommend that the underwater measurements be performed in conjunction with a similar unit mounted on gimbels, in a position free from shadows and strong reflections, and preferably during the middle of the day. If light attenuation measurements are made in the early morning or late evening, then the attenuating coefficients may be overestimated by as much as 25%, due to sun angle effects. The underwater unit should be suspended as far outboard as possible on the sunny side of the ship and should be maintained in a vertical position.

Measurements taken over a series of depths are normalized to a constant value of the deck unit and are plotted on 3-cycle semi-log paper to obtain a good estimate of the diffuse attenuation coefficient K (m^{-}):

$$\frac{K_{Z_1+Z_2}}{2} = \frac{1}{Z_2 - Z_1} \ln \frac{I_{Z_1}}{I_{Z_2}} = \frac{2.3}{Z_2 - Z_1} \log \frac{I_{Z_1}}{I_{Z_2}}$$

Where Z = depth in meters, I = irradiance, and y = specific relative irradiance. Depths of specific relative irradiances are then calculated:

$$Z_{Y\%} = \frac{4.605 - \ln Y\%}{K}$$

or determined graphically.

In the event that a submarine photometer is not available, a Secchi disc may be used to provide a very rough estimate of the diffuse attenuation coefficient. The general formula for determining the attenuation coefficient (K) from Secchi disc depth (D) in meters is:

$$K = \frac{2.0}{D}$$

Values of K estimated from Secchi disc depths may easily be in error by 25%, depending on a variety of factors including the amount of backscattering of light by surface water and roughness. We suggest that it be on hand but used only as an emergency measure if the submarine photometer fails.

B. WATER SAMPLER

It is important that seawater be collected with a non-toxic sampler. In no case should metal come in contact with water to be used in production measurements. A sampler such as the opaque-plastic Niskin bottle is ideal since it has no exposed metal. Also, it is darkened and will prevent exposure of phytoplankton to high light intensities as it is brought aboard ship.

Niskin bottles should be secured in a shaded area on ship. When water is drawn to fill glass productivity bottles, larger zooplankton should be removed by filtration through a plastic funnel that contains 200 μm mesh Nitex netting. Glass bottles should be covered with a black cloth bag to shade phytoplankton before they are placed in incubators.

C. INCUBATORS

To optimize expensive ship time, it is desirable that incubations be carried out on ship and not in situ. Actually, little bias appears to be induced by incubating samples under neutral density screens as opposed to an in-situ incubation. Holmes (1968) compared the in-situ (in seawater) method with productivity samples held under fluorescent light or natural sunlight with shading by neutral density filters. He concluded that the latter two methods gave unbiased estimates of actual in-situ production.

We recommend the simultaneous use of two types of incubation. One set of productivity bottles should be held in an artificial light box with shading by neutral density screens. This system allows the incubation of samples under a known, and constant, light source. A second set of bottles is incubated on deck in sunlight. Incubation in sunlight allows a measure of the degree of inhibition of photosynthesis from ultraviolet and high light intensity which is not possible under fluorescent light. It also more closely approximates natural conditions.

As mentioned previously, in short-term experiments there seems to be no bias induced by using neutral filters rather than colored filters which would duplicate the light found at depth. However, it should be noted that in long-term incubations the quality of light can affect the nature of the photosynthate (Wallen and Geen, 1971). Recently Shimura and Ichimura (1973) measured photosynthesis in the northwestern North Pacific phytoplankton under blue, white (fluorescent), red and green light for 3-hour incubations. They noted that the photosynthetic efficiency in green light was 80 to 90%, and that of blue light was 105 to 115% of white (fluorescent) light, respectively.

Gray-plastic window screening, which is available at most hardware stores, can be used to attenuate light. We have observed the following relationship between layers of screen and light attenuation:

Layers	% Light
0	100
1	60
2	40
4	16
6	6
9	1.5

Due to variations in manufacture of screening, investigators must check the light reduction characteristics of their own screens. To diffuse light entering the bottles, one layer of a thin-white, translucent-plastic sheet can be placed between the screening and the Plexiglas tubes.

Artificial Light Incubator

Primary productivity estimates frequently must be made during overcast conditions. In order to make meaningful inferences about the average productivity of an area, variations brought about by changes in daily irradiance must be taken into account. The extrapolation of productivity estimates made at low irradiance levels (as on cloudy days) to high irradiance levels (as on sunny days) is difficult because the productivity-irradiance relationship is curvilinear and because acclimation can result in varying slopes and saturation intensities within populations. However, it is possible to make comparative measurements using artificially illuminated incubation boxes which expose the samples to a constant, saturating irradiance. This is the major reason for incubating samples in an artificial light box.

The use of "daylight" fluorescent bulbs gives a closer approximation to the spectral quality of natural daylight than incandescent bulbs. The latter emit predominantly red light, but usually produce a lower overall irradiance level. It is very important to include a description of the light source as well as the irradiance experienced by the samples. This irradiance should be measured with a detector of the type described above. The entire artificial illumination incubator should be screened from unwanted sun and laboratory light.

Artificial illumination incubators also permit one to measure the potential productivity of samples collected at all hours of the day. However, care must be taken to account for diel rhythms in light and dark CO_2 uptake capacity if these results are to be used to estimate the productivity of a given area. For example, at one station occupied over a 24-hour period from noon to noon, we found that nighttime light bottle uptake, as measured in an artificial illumination incubator, was twice that of daytime values. Dark bottle uptake increased by an order of magnitude during the dark period. The variation in chlorophyll *a* concentration was less than 20% during the 24-hour period.

Probably the simplest type of incubator suitable for artificial light incubation is that based on a design by John Ryther (personal communication). This consists of Plexiglas tubes covered with various neutral density screens to attain the desired light attenuation. The glass productivity bottles are slipped into the tubes, and ends of tubes are sealed with rubber stoppers. Each rubber stopper contains a piece of hollow plastic pipe for the passage of seawater from the ship's deck-wash system. The whole assembly is mounted at a 45° angle to a bank of fluorescent lights. Cooling seawater flows in from the bottom and out the top and is distributed to the tubes from a manifold arrangement. Temperature of the flowing seawater should checked regularly to assure that it is the same as that of surface water.

Other incubator designs can be used to obtain good production data. For comparative purposes, we illustrate the incubator designed by Fee (1973) in Figure 1. Fee successfully used this incubator in a production study on Lake Michigan. Samples were incubated for 4 hours and agitated by rotation. In the open ocean if the incubation times are short (ca <4 hr) the bottles need not be rotated.

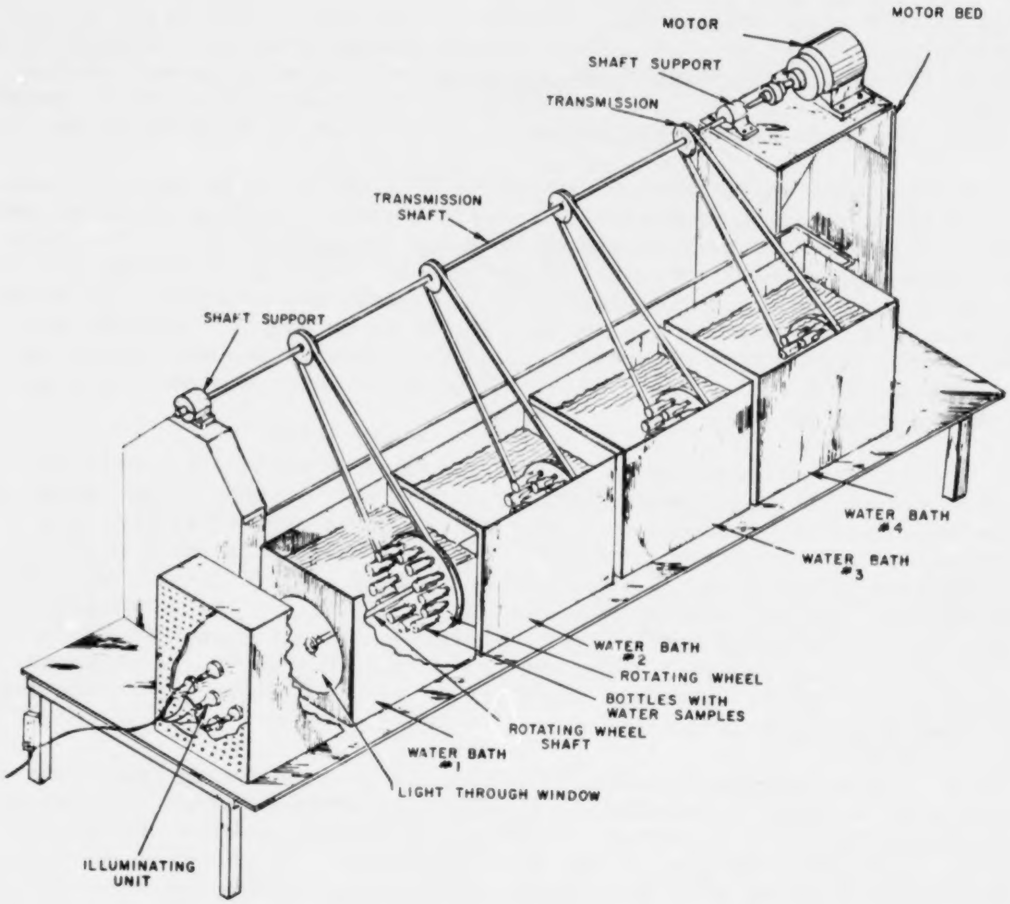
MOTOR
MOTOR BED
SHAFT SUPPORT
TRANSMISSION
TRANSMISSION SHAFT
SHAFT SUPPORT
WATER BATH #4
WATER BATH #3
WATER BATH #2
ROTATING WHEEL
BOTTLES WITH WATER SAMPLES
ROTATING WHEEL SHAFT
WATER BATH #1
LIGHT THROUGH WINDOW
ILLUMINATING UNIT

Figure 1. Incubator used by Fee (1973) for measuring phytoplankton production in Lake Michigan

The light source for this incubator was a bank of six 500-w T3Q tungsten iodine vapor bulbs. Each bulb was backed with a paraboloid reflector of brightly buffed aluminum to direct maximum amount of light toward the experimental changers. Tungsten-iodine bulbs emit very high irradiances and have a very long life with little change in the quality of light emitted. The heat released by the bulbs was largely removed by placing a rectangular Pyrex box directly in front of the first chamber. The box was 5 cm wide and was filled with rapidly circulated cold water.

Ambient Light Incubator

The outdoor incubator which is exposed to natural sunlight can be considerably simpler than the artificial light incubator. It is possible to simply place bottles in a rectangular box with five compartments in line.

There are holes in partitions between compartments so that seawater from the ship's deck-wash can flow in one end of the box and out the other. Each compartment is covered with a different number of neutral-density screens to attain the desired light attenuation. The box is shallow so that there is little attenuation of UV light as a result of passage through seawater. Compartments receiving 100% of surface light contain plastic bottles which permit passage of UV light. Those with 60% light and less contain glass bottles which absorb UV. This system approximates the depthwise penetration of UV light in the sea.

Bottles with phytoplankton exposed to UV light could be made of quartz glass, but these would be expensive. Instead of quartz, bottles could be made of acrylic plastic. Ordinary Plexiglas will not transmit UV light, but those made of polymethyl-methacrylate will allow passage. This is available as 3 mm thick PMMA Shinkolite A acrylic plastic by Mitsubishi Rayon, Japan. In using this plastic with shallow bottles (90 mm dia. by 25 mm deep), Ilmavirta and Hakala (1972) observed that production in near-surface waters was reduced by 50% as compared with measurements made in Jena glass which excluded UV light. Tests were carried out in a lake in Finland.

The optical attenuation of the neutral density screening should be checked by placing the underwater detector within each chamber. The inside of the chamber should be painted with flat-black paint to prevent reflections from the walls and bottoms, or flat-dark blue to simulate the small contribution of upwelled light in situ. The chambers should be sufficiently wide to prevent shadows from the wall from falling on the bottles. Alternatively, screening can be placed around individual bottles that are placed within a lucite box of flowing seawater. The bottoms of this box should be darkened for reasons cited above. The deck units should be situated in a position free from shadows and strong reflections.

Continuous irradiance measurements should be made during the incubation period to express production as a function of light exposure (irradiance x time). A relatively inexpensive, self-contained solar recorder is made by the Belfort Instrument Company of Baltimore, Maryland. Output from the solar recorder can be compared with the submarine photometer, which has an output of einsteins $cm^{-2}sec^{-1}$, to obtain very rough calibrations.

D. DURATION OF INCUBATION

In the ambient and artificial light incubator, bottles should be exposed no more than 3 hours. In the sea, phytoplankton move from one light intensity to another as they drift with currents. Thus it would be unnatural to incubate them for relatively long periods at one light intensity. In clear, open ocean water, light penetration is quite deep and a 3-hour incubation of these phytoplankters at one light intensity is probably not unnatural. However, in some estuaries phytoplankters may regularly move from the 1% to 100% light intensity in a matter of minutes. Prolonged incubation at one intensity might yield a biased result.

There are other reasons for avoiding long incubations. For example, Vollenweider and Nauwerck (1961) have observed that the production sum of three or four short incubations exceeds the total of one long exposure. Also, Harris and Lott (1973) have shown that long exposures of phytoplankton to high light intensities leads to an increase in photorespiration. Finally, another reason for avoiding long exposures is that glass ampules, the usual method for storing ^{14}C, bicarbonate, can add sufficient silica to stimulate production over long incubation periods (i.e., 24 to 28 hours). Gieskes and van Bennekom (1973) noted up to 1 mg-at liter^{-1} of dissolved silica in ^{14}C stocks stored in distilled water in glass ampules. They also noted that if incubation times are relatively short there is no simulation in productivity as a result of silica enrichment. All of these considerations indicate that duration of incubation should be as short as possible.

E. ^{14}C BICARBONATE STOCKS

We and others have experienced problems with certain manufacturers concerning the quality of ampulated ^{14}C stocks. Some have contained toxic material, others particulate matter (Morris et al., 1971), and all are likely to contain high silica concentrations (Gieskes and van Bennekom, 1973). We suggest that ^{14}C bicarbonate stocks should be purchased as a crystalline solid and ampulated by the investigator. An alternative, as suggested by Morris et al. (1971), is to pool the ^{14}C from all the ampules. The pooled ^{14}C should then be UV-irradiated to remove organic matter that is associated with ^{14}C. Additionally, a "zero time blank" should be made by adding the normal concentration of ^{14}C to a 125 ml glass incubation bottle containing unfiltered seawater (Morris et al., 1971). The bottle contents are immediately filtered and the activity of this filter is substituted for dark-bottle uptake. Morris et al. (1971) recommended that this be used as the standard dark uptake correction. We agree, but only if the uptake in normally incubated dark bottles exceeds 10% of the uptake in light bottles.

If the investigator chooses to dilute and ampulate his own crystalline ^{14}C, then care should be taken that the samples are clean and free of particulate matter. Sodium chloride should be added to distilled water to give a salinity of 35 ppt and the pH should be adjusted to 9.5 with dilute NaOH solution before adding crystalline ^{14}C to the solution. Aliquots of the radioactive solution are added to samples which should be immediately sealed and autoclaved. To test for improper sealing, the ampules can be placed top down in a solution of dye while being autoclaved. If not sealed, ampules will sink after autoclaving and dye will be present within them.

One convenient way to prepare the ^{14}C solution is to anticipate how many samples will be required per station and then place the total concentrated amount as, say, 5 ml, in one ampule. At the station, this volume can be diluted with filtered seawater, and one ml of the diluted stock solution can be added to each bottle with an automatic pipet. This system saves time and money since glass ampules are expensive, and insures that a uniform amount of ^{14}C is delivered to each bottle.

Generally, 10 to 20 μCi of ^{14}C bicarbonate should be added to each bottle in oceanic waters and from one to 5 μCi in estuarine regions.

F. DETERMINATION OF ^{14}C UPTAKE

Many difficulties regarding self-absorption and standardization of ^{14}C stocks arise when the thin-window gas-flow beta is used. The scintillation counter method allows relatively easy standardization of stocks and efficient counting of samples (Schindler, 1966).

After incubation, the entire contents of a 125 ml bottle should be immediately filtered. Caution should be observed if formaldehyde is added to the bottle to terminate carbon uptake. Ilmavirta (1974), working in Finnish lakes, observed an average 21% decrease in the activity of samples only five minutes after the addition of formaldehyde. However, he did not state whether the formaldehyde was buffered or how much was added.

Arthur and Rigler (1967) have observed that, as the volume of sample filtered increases, phytoplankton are damaged and cell contents are lost through the filter. The authors used 25 mm diameter Millipore® HA filters with a maximum pressure differential of 300 mm Hg. However, MaMahon (1973) noted that the retention capacity for ^{14}C by the filter, expressed as radioactivity per ml filtered, is maximum for small samples ($\leq$ 1 ml) and decreases to a constant value when samples are larger than 100 ml. This is attributed to absorption in the filter and probable retention of ^{14}C bound to unknown substances in the water or on the filter, which are eluted or exchanged by passage of volumes of water of 100 ml or more. The error can simply be corrected by washing the filter with a few ml of nonradioactive filtered seawater before and after the bottle contents are filtered. Allen (1971) recommends against washing with dilute HCl since acidification removes a significant fraction of previously incorporated carbon.

Another problem with the Geiger-Mueller counting method is that phytoplankton penetrate the filters, thus increasing problems of self-absorption (Theodorsson, 1975). This problem is also eliminated if the scintillation counting method is used.

Yet another apparent error was demonstrated by Wallen and Geen (1968) who noted that desiccation of algae in filters before addition of filters to vials containing scintillator fluor could lead to losses of up to 50% of activity. In spite of this observation, Lind and Campbell (1969) recommended drying the filters since good water-accepting scintillation fluors were not available. Ward and Nakanishi (1971, 1973) found that the loss of ^{14}C during desiccation increases with decreasing incubation light intensity. They compared Geiger-Mueller with liquid scintillation methods for counting filters and found that, when production was calculated on an aerial basis, results of the latter method generally were 25-40% greater. A good water-accepting naphthalene-dioxane fluor was developed by Schindler and Holmgren (1971) for use with samples from freshwater lakes. However, its use might be limited in saltwater, since any variation in the salt retained by the filters can cause counting to be erratic (Pugh, 1973). The xylene-based fluor, Aquasol®, can be used, but it is costly. If Aquasol® is used to count bicarbonate standards, phenethylamine should be added to 10-20% v/v final concentration to eliminate loss of ^{14}C due to the low pH of the cocktail (Iverson et al., 1976). This precaution can prevent errors up to 40% in knowledge of the initial activity.

In addition, the high chemiluminescence of the Aquasol® system must be considered when counting plankton samples. This potential error can be eliminated by counting samples only after they have remained in the fluor for about a week. The ^{14}C counting methods of Pugh (1970, 1973) seem to offer the most reliable results in assaying ^{14}C retention by marine phytoplankton. Pugh (1973) suggests the use of cellulose nitrate (Sartorius Membrane-filter MF 125) filters or PVC filters since they decompose more completely in a toluene fluor than do cellulose ester filters. Pugh (1973) obtained best results when he used 2-methoxyethanol-toluene fluor. This consists of 1:2 v/v 2-methoxyethanol: 0.5% Butyl PBD in toluene. Using the cellulose nitrate filters and the toluene-based fluor, Pugh (1973) did not observe any apparent loss of radioactive material as the filtered volume was increased. Further, there was no loss of activity when the filters were dried and stored overnight at 70°C. Also, wet filters could be added to the fluor if necessary and counts could be made after several weeks with no loss of activity. The scintillation cocktail Aquasol® was not tested by Pugh (1973).

In using the scintillation counter, Pugh (1970, 1973) recommends the use of the filter standardization method for counting intact filters. Cells and membrane filters have a self-absorptive effect that is not corrected by external standardization. It is especially important to standardize by this method since phytoplankton are known to penetrate membrane filters rather than lie on the surface as a thin film (Theodorsson, 1975). The filter standardization method, however, is only accurate when the weight of algae on the filters is small (<1 mg dry wt). It would be highly unusual to have more than one mg of algae material in 125 ml of oceanic water. The techniques involves construction of a quench curve relating channels ratios to counting efficiency. Scintillation counts are recorded in two channels. One channel counts only weak β activity, while the other channel records all the activity in the ^{14}C β spectrum. The ratio of counts in the first channel to those in the second is called the *channels ratio*. As described by Pugh (1973), the investigator first filters natural phytoplankton and assembles several filters, each with a different amount of plankton. The volume filtered may vary from one ml to one liter. Next, approximately 0.1 ml of a ^{14}C sucrose solution is micropipetted onto the filters and then allowed to dry. Sucrose is used because it is virtually insoluble in the toluene fluor and will remain on the filters. Filters are placed in scintillation vials, completely covered with fluor solution (10 ml of 0.5% Butyl PBD in AR Toluene), and counted. As described, counts were recorded on two channels set for optimum of a homogeneous solution. From these counts the channels ratios were calculated. The specific activity of the original ^{14}C sucrose solution is determined by counting 0.1 ml samples in homogeneous solution in a toluene:ethanol (3:1) fluor; from the computed *channels ratio*, the specific activity can be calculated from the standard curve for the scintillation counter. The result is a quench curve (Fig. 2) that takes into account the self-absorptive properties of phytoplankton and filter.

An alternative to the filtration technique of removing phytoplankton from unassimilated ^{14}C is bubbling (Schindler et al., 1972). In this technique, about 20 ml from the incubation bottle is pipetted into a 30 ml tube with fine-fritted glass at the base (Fig. 3). The pH is lowered to between 3 and 3.5, and air is bubbled through the column for about 20 minutes. This removes inorganic ^{14}C from solution, and the remaining ^{14}C is that contained in the cells or which has been excreted and is organically bound. Next the investigator removes 2.5 ml from each filter tube and places it in a scintillation vial with fluor for counting. Since there is no filter, there is no absorption problem and the external standardization channels ratio can be used for calibration. The beauty of this technique is that there is no problem with cell rupturing when filtering. At times, Schindler et al. (1972) noted that ^{14}C uptake with the bubble method was twice measured by filtration.

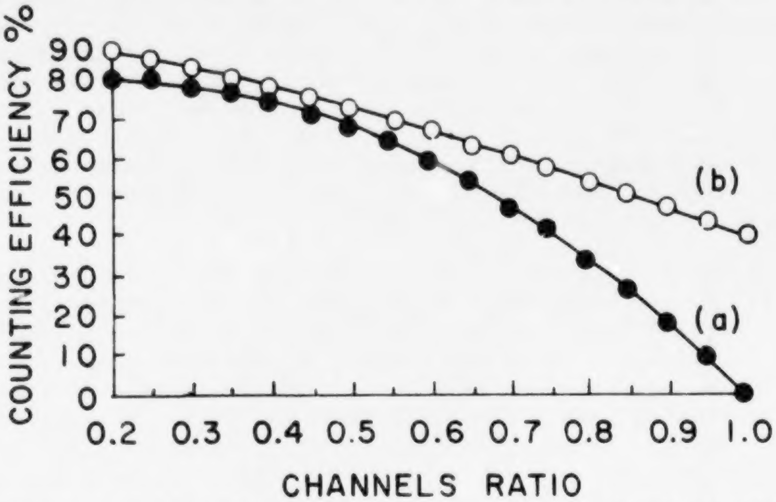

Figure 2. Channels ratio calibration curves. Filter standardization curve (a) calculated as a second order polynomial from 220 points ($r = 0.970$). Channels ratio curve (b) is the standard curve for counting in a homogeneous solution. From Pugh (1973).

However, several workers have indicated that this method yields highly variable results. It is possible that some fraction of labeled excreted organic material is hydrolyzed and converted to $^{14}CO_2$, which may be purged from the solution under these conditions and is therefore not counted (Iverson, personal communication), leading to underestimates of phytoplankton carbon fixation if excretion is significant.

G. DARK UPTAKE

The problem of high uptake of ^{14}C in dark bottles has been encountered by several workers. Generally, dark uptake of ^{14}C should not exceed 10% of uptake in the light. However, Holmes (1968) reported that in the open Pacific dark uptake occasionally equalled uptake in the light. Holmes had no adequate explanation for high dark-bottle uptake but thought that perhaps an occasional ampule containing particulate radioactive material may have been the cause. High dark-bottle uptake was also encountered by Steven (1971) while working in the western tropical Atlantic. High dark-bottle counts occurred through the year, often exceeded light-bottle uptake, and were present at all depths sampled. The problem was solved by making all collections at sunrise and completing the experiments with minimum delay. When this procedure was adopted, dark-bottle counts dropped to less than 6% of counts in light bottles.

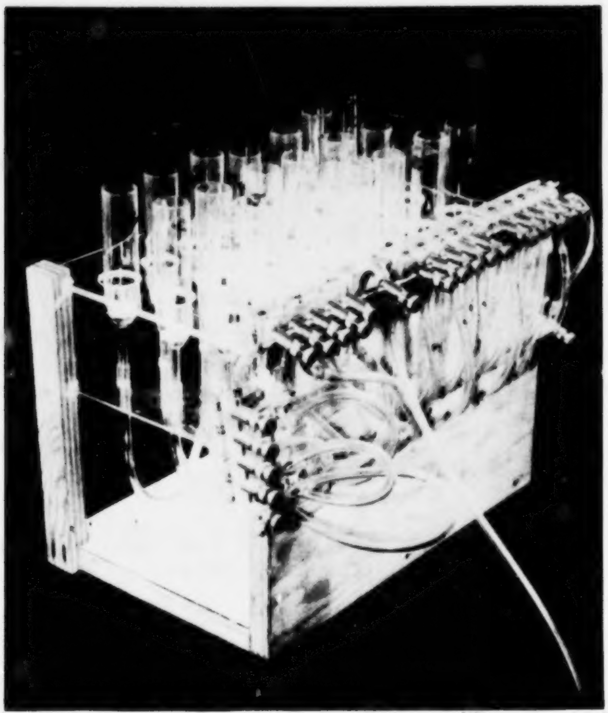

Figure 3. Apparatus used to drive off inorganic ^{14}C in productivity measurements. From Schindler et al. (1972).

Morris et al. (1971) observed in the field and in laboratory cultures that high ^{14}C uptake in dark bottles was associated with low phytoplankton cell densities. For example, Figure 4 shows that the ratio of light carbon dioxide fixation to that in the dark is high in relatively dense phytoplankton culture. At lower cell densities, dark-bottle fixation increases in relation to light-bottle uptake until uptake in both light and dark are equal. Morris et al. (1971) had no adequate explanation for this phenomenon. Until more is known concerning the mechanism of dark fixation, they recommend that it be ignored. In this case the blank would be the time-zero control as described under the heading "^{14}C bicarbonate." At present, there seems to be no adequate explanation for high dark-bottle counts. Perhaps this actually represents carbon incorporation by bacterioplankton (Sorokin, 1971), although this theory has been criticized (Banse, 1974). At present we must agree with Yentsch (1974) who, in speaking of high-dark uptake, stated, "This is another one of those indefinable mysteries which pervade the discipline."

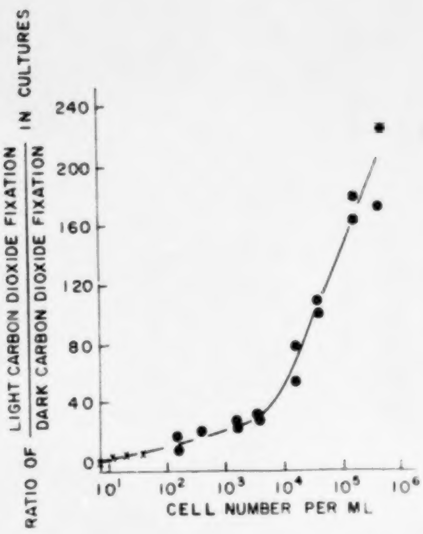

Figure 4. Relationship between cell number per milliliter (log scale) and the ratio of light CO_2 to dark CO_2 fixation in cultures of *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. From Morris et al. (1971).

H. CALCULATION OF PRODUCTION

The basic formula for calculating marine primary production has been presented by Strickland and Parsons (1972). This is:

$$\text{mg C/liter/hr} = \frac{R_s - R_b \times W \times 1.05}{R \times N}$$

In the formula, 1.05 is a factor used to correct for the fact that the heavier ^{14}C isotope behaves differently from the ^{12}C found in nature. N equals the incubation time in hours. W is the weight of inorganic carbon available to the algae. Total ^{14}C added per bottle is represented by R. Carbon-14 retained by the light- and dark-bottle filters are R_s and R_b, respectively. The formula should be modified if the acidification-bubble technique is used or if only part of an incubation bottle is filtered. It is assumed that there is about 90 mg CO_2 liter^{-1}, and in oceanic waters this would range between 85 and 103 mg liter^{-1}. For measurement of available CO_2 in low salinity waters, one can use the techniques described by Strickland and Parsons (1972) or Rao (1965).

As Fee (1969, 1973) has pointed out, the interpretation and expression is difficult. Because of problems of extracellular excretion and respiration, it is necessary to confine incubation times to short intervals. Yet, daily rates are needed for a reliable estimate of annual or seasonal production. There are problems in extrapolating rates measured in natural sunlight in sunny weather to cloudy days. A computer model has been constructed by Fee (1969, 1973) to allow the calculation of total daily photosynthesis. The model is available in Fortran IV language (Fee, 1971). As inputs to the model, the following are required: (1) shape of the curve relating photosynthesis to irradiance (P vs I curve), (2) highest photosynthetic rate, (3) extinction coefficient of seawater at sample site, and (4) time variation of photosynthetically active illumination entering through the water surface over the interval of interest, usually a day. The model does have some limitations. For example, it does not take into account the "afternoon depression" of photosynthesis. However, it has the advantage of allowing a large number of stations to be processed rapidly, and it appears to closely approximate in-situ production.

The quantum yield approach developed by Bannister (1974) for nutrient-saturated layers may represent a significant advancement but has not yet been applied to oceanic situations and again does not adequately treat photo inhibition or the afternoon depression.

In summary, it is apparent that there have been numerous technical problems associated with the ^{14}C light-dark bottle method. However, recent advances, mentioned in the text, can alleviate many of these biases. Most of the errors noted in the literature lead to underestimates of oceanic production. As a guess, it would appear that, due to these biases, actual oceanic production, may be underestimated by as much as a factor of 2 or 3. Further research and improvements in the ^{14}C light-dark bottle method by both Soviet and American oceanographers will undoubtedly yield a more accurate estimate of primary production in the open sea.

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RELATIVE ABUNDANCE OF SYMPATRIC SPECIES AND MODEL OF EXPONENTIALLY BROKEN ROD (EBR)

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In 1957 Robert MacArthur (MacArthur, 1957) considered three alternate versions of the possible distribution of N individuals among w species, and demonstrated good agreement between the distribution of trophic birds and one of these versions. This case corresponds to the initial assumption that in a relatively homogeneous biotope, groups of closely related species which are comparable by size and physiology retain a stable population ratio, i.e., their relative abundance turns out to be constant and can be predicted by some model.

Formally, this case is analogous to a segment of a rod of unit length (corresponding to the biotope) which is partitioned into w parts by w-1 points falling randomly on it. At the points of impact, the rod is broken into parts whose lengths are proportional to the population sizes of the individual species, so that the mean sizes of the parts form the same ratios as the numbers

$$\frac{1}{w}, \frac{1}{w} + \frac{1}{w-1}, \frac{1}{w} + \frac{1}{w-1} + \frac{1}{w-2}, + \dots + \frac{1}{w} + \frac{1}{w-1} + \dots . \quad 1$$

The expected abundance of the r-th species among N individuals and w species can be predicted using the formula

$$\hat{n}_r = \frac{N}{w} \sum_{i=1}^{r} \frac{1}{w-i+1} \qquad (1)$$

where $\hat{n}_r$ is the length of the segment having the number r in the sequence of populations ranked by size from 1 to w. Biologically, this model, named the "broken rod model" by biologists (henceforth referred to in this article as the "B.R." model), corresponds to the initial assumption that in a system in equilibrium the niches of sympatric species are contiguous without considerable overlap.

In the same year, G. Hutchinson (Hutchinson, 1957), using the concepts of set theory, determined elegantly the fundamental niche. He also demonstrated that the non-overlapping segments predicted by the B.R. model have a great deal in common with the concept of a fundamental niche reflecting the total ecological requirements of the species.

The non-overlapping of niches in higher dimensional space discovered by MacArthur presupposes unequivocally the absence of interspecies competition, since otherwise, according to the Gauze-Volterra axiom, the relative abundance of species could not be constant, i.e., they could not form population size ratios which are stable over time in a homotypic association (what is meant is similarity in regard to type of feed). Moreover, species in the same trophic grouping are forced under similar conditions to a similar type of existence, which in turn inevitably leads either to a local crowding out of species, or a modification of the ecological requirements of one or perhaps even all species in ways which will tend to reduce competition among them. Feed selectivity, different resistance to variations of different variables in the biotope, and finally, rivalry for shelter refine the relationship between partners and thus turn out to be the causes responsible for the creation and support of stable associations of sympatric species.

I deliberately avoid mentioning the most trivial mechanism supporting this organization--the competition for food, which depends on the density of the individuals N. The competition for food forces the partners to make concessions, which force the "opening up" of various sections of the genome "line," so that the diversity of requirements and phenotype adaptation can avoid an overlapping of fundamental niches with respect to a scarce component, and consequently the competitive crowding out of species. As a result, in a system in equilibrium (for the case stipulated by the B.R. model), individual species do not vanish and no species attains a population size which entails a destruction of the ecological niches of other species. Therefore, not only the fact that the population size ratios among the species making up the system remain constant, but also the absence of fluctuations in the total density of individuals in the association can be justifiably considered as a characteristic of a system in equilibrium.

It was by no means coincidental that MacArthur considered those species to be in equilibrium for which the integral in the equation

$$\log N_{i(t)} = \log N_{i(0)} + \int_0^t r_{i(t)}\, dt \tag{2}$$

was smaller than the logarithm of the original number of individuals, where r_i is the population growth rate of the i-th species (MacArthur, 1960). Somewhat later, Preston (1968) considered a species to be stable if the variations in its population size (both in time and space) over many years obeyed a lognormal distribution.

Thus, for the case stipulated by the B.R. model, the co-existing sympatric species must be in equilibrium. The mutual interdependent partitioning of the segment by means of randomly falling points reflects only the fact that the spacing of the niches is not discrete, which in no way implies that one species becomes more abundant at the expense of crowding out another species. Rather, the situation is such that several related species located in an unoccupied but *confined biotope* "partition" the latter in such a way that no free gaps are left between their niches. This final result is achieved as a consequence of the action of mechanisms regulating the ecosystem, whose purpose is to compensate the activities of each individual population by the collective activities of all other populations from various trophic groups utilizing jointly the available biotype resources.

While the competitive crowding out mechanism can only occur as an episodic event, the functional equilibrium of the ecosystem is disturbed whenever the total density of individuals in the sympatric species increases (or due to any other cause leading to a functional imbalance of the ecosystem).

Returning to the B.R. model, we note immediately that it describes a special case. The initial restrictions are so strict (sympatric equilibrium species of approximately the same size, with a constant total density, confined to a relatively homogeneous biotope) that it is actually surprising that in certain cases the agreement between the prediction made by MacArthur's model and reality is satisfactory. Meanwhile, the great popularity enjoyed by MacArthur's "broken rod" model among ecologists is attributable precisely to the good agreement detected between its forecasts and the observed relative abundance of sympatric species in certain associations of small animals (ciliary Ophiuroidea, Gastropoda, hermit crabs, snakes and certain fish). The most exhaustive "biological" analysis of the broken rod model is due to Ch. King (1964) to which we refer the reader interested in the details of the problem. The range of w and N values for which data showing good agreement with the forecasts made by the model were reported varied from 5 to 30 species and from 20 to 200 individuals. At the same time, the study of phytoplankton undertaken by Hutchinson (1958, 1961) showed poor agreement with forecasts made by the B.R. model. I demonstrated during a study of seasonal variations in offshore marine plankton that the B.R. model predicts the relative abundance of species in a comparatively large proportion of cases when the total number of individuals is not large (Fedorov, 1970). On the other hand, during the spring and fall phytoplankton bloom period, the deviations from forecasts made by the model are not only substantial, but they also obey a particular law, namely, the most common species are more abundant and the rarest species less abundant than the values predicted by the model. Ultimately, this observation served as the main impetus for a revision of the original assumptions and hypotheses discussed by MacArthur (1957). To begin with, a fact attracting attention is that in the B.R. model the ratios of the lengths of the parts depend only on the number of points falling on the rod (i.e., the number of species w) without being dependent on N. This means that MacArthur makes no allowances for the effect of the total density of individuals on the population sizes. This can only occur in the absence of competition for food and the competitive crowding out of individuals. At the same time, a more general model of the relative abundance of sympatric species including MacArthur's B.R. model as a special case (corresponding to the absence of competition for food), must certainly also include an additional variable which is related to the total number of individuals in potentially competing species, i.e., to N. While retaining the formal analogue of the model (partitioning of a rod into parts which are proportional to the expected average population sizes), the main difficulty of the problem is carried over to the plane. The problem which arises is what function must be used to specify the non-uniform distribution of the points on the rod when N varies.

A GENERALIZATION OF THE MODEL OF THE RELATIVE ABUNDANCE OF SPECIES

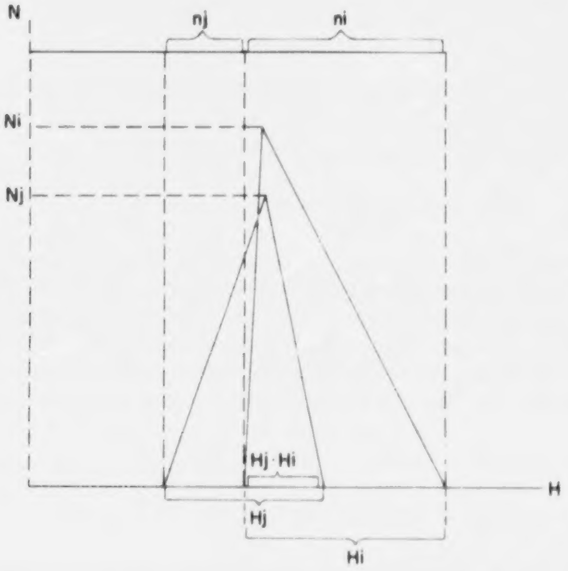
Among all possible prerequisites, we single out three which are of prime importance in the construction of a more general model.

1. In terms of the concepts of D. Hutchinson's formal theory, the intersection subset $(H_i \cdot H_j)$ formed by niches overlapping with respect to a number of identical parameters is always *smaller* than the potential niche size (H_i, H_j) of any species, since species with niches that are completely identical cannot exist in a group of sympatric species. In other words, the intersection subset formed is related to the size of the fundamental niche in the same way as the phenotype is related to the genotype. Consequently, the competitive crowding out from the biotype region (corresponding to the intersection subset of one species) to the region which corresponds to the "refuge" of the crowded species $(H_i - H_i \cdot H_j)$, leads to phenotypic changes in the latter not allowing one species to crowd out another completely (due to the formation of new "cells" in the genome line). With respect to specific structural characteristics of their niche, the crowded species which form an ecological reserve of the association, are most closely related to the crowding species. A study of diatomaceous plankton in the White Sea has shown that the most common and rarest species react similarly to conditions in experiments conducted in situ, whereas "neighboring" species in the sequence of populations ranked by size react in the most dissimilar manner.

2. The intersection subset $H_i \cdot H_j$ turns out to be a region in which the Volterra-Gauze axiom admits no exception. Consequently in such a system, the population sizes can only stabilize after one species is crowded out from the locus corresponding to the intersection subset, i.e., after the biotope is partitioned between the crowded and crowding species according to the condition $H_i - H_i \cdot H_j$ and $H_i + H_i \cdot H_j$ respectively.

3. The region of intersecting subsets increases with an increase in the total density of individuals N, since the total demands on the environment made by the entire association as a whole exceed the capabilities of the biotope at every instant of time. Therefore, competitive crowding processes shift the population size ratio in favor of the crowding species (see prerequisites 1 and 2), and due to this mechanism the most common species become more abundant and the rare species which are crowded into shelters, less abundant than predicted by MacArthur's model.

A rod of finite length (0,1) can again be used as a formal analogue of the more general case described above. Suppose that the potential size (not the population size!) of each species is determined by a pair of points falling randomly on the rod from a height which is proportional to N. Clearly, on the average, the increase in the distance between the falling points will be proportional to N if the surface of the rod is elastic (see Fig. 1). Consequently, the probability that the niches will overlap, i.e., that a region of intersecting subsets will be formed, will also increase. As a result of competitive crowding, one species is crowded out by another species from the zone $H_i \cdot H_j$, so that the segments $(H_i + H_i \cdot H_j)$ and $(H_i - H_i \cdot H_j)$ obtained are proportional to the population sizes of the sympatric species n_i and n_j respectively (not their potential sizes). Moreover, it is evident from Figure 1 that the length of the overlapping part corresponding to the intersecting zone must be subtracted from the segment corresponding to the requirements of the species according to some rule (for example, the common part can always be subtracted from the right segment, or to take another example, the smaller segment can always be subtracted from the larger segment). Due to this mechanism which changes the lengths of the segments with increasing N (and consequently also the intersecting zones ($H_i \cdot H_j$)), *all segments except one* will become increasingly smaller and the largest segment will approach unity. Since the first prerequisite $N_i \ngtr N_j$ in principle precludes the elimination of shelters even by the most competitive species, when the number of species remains approximately constant ($W \approx \text{const}$), with increasing N the decrease in the abundance of individuals in the sequence of populations ranked by size will follow an exponentially decreasing curve.



Key: a. i.e., for $N_i > N_j$ and $n_i > n_j$

where $n_i = H_i + n_i \cdot H_j$ and $n_j = H_j - H_i \cdot H_j$

Figure 1

Mathematically, the observed pattern corresponds to assuming in the broken rod model that when w-1 points fall on the rod, the probability of a point making impact at different ends of the rod is not the same, and that it differs by a value (which is measurable along the rod) which varies from "$-\mu$" to "$+\mu$" at its ends.

In view of the exponential character of the curve describing the decrease in the relative abundance of species during competitive crowding, the logical and simplest assumption is that the (w-1) points falling on the rod follow some non-uniform distribution which is proportional to $e^{\mu x}$, where μ is an unknown parameter. Hence "μ" is an important parameter reflecting the change in the ratio of population sizes of species, which depends on competition for feed (or niches?). Competition becomes more important as the total density of individuals N increases. Moreover, when $\mu = 0$ (i.e., in the absence of competitive crowding out of species), any possible model must degenerate to the special case specified by MacArthur's B.R. model.

EXPONENTIALLY BROKEN ROD MODEL (E.B.R.)

Ya. I. Gol'fand performed by no means trivial mathematical calculations which made it possible to select the parameter μ. A full justification of these calculations is given in a supplement to the study dedicated to the "Ecology of Phytoplankton" (to be published). Using the former formal analogue of a broken rod, essentially the generalized model reduces to the following:

A rod of unit length is broken into w parts. It is assumed that the (w-1) "breaking" points are independent random variables which are identically distributed on the interval (0,1), with the density

$$\rho(\alpha) = \mu/_{\sinh\mu} \exp\{\mu(2\alpha - 1)\} \tag{3}$$

where μ is a parameter and $\mu/_{\sinh\mu}$ is a normalizating factor. We obtain the ranked sequence by ordering the parts after the rod is broken in a decreasing sequence. This sequence is random, since it is associated with the corresponding random breaking of the rod. The problem reduces to finding the expected values of random variables in ranked sequences. These quantities are functions of the parameter μ, so that they can be written as

$$1 > n_1(\mu) > n_2(\mu) > n_3(\mu) \ldots > n_w(\mu) > 0 \tag{4}$$

$$n_1(\mu) + n_2(\mu) + n_3(\mu) \ldots + n_w(\mu) \equiv 1 \tag{5}$$

When $\mu = 0$, the breaking points obey a uniform distribution ($\rho(\alpha) \equiv 1$), reflecting a special case of the model, i.e., MacArthur's B.R. model.

$$\hat{n}_r = \frac{1}{w} \sum_{k=r}^{w} \frac{1}{k} \tag{6}$$

In this formula, the order of the numbering sequence was changed (compared to formula 1), and the most abundant species has rank 1.

In the case where $\mu \neq 0$, the exact formula for $\hat{n}_{r(\mu)}$ is considerably more complicated

$$\hat{n}_{r(\mu)} = \int_0^1 k_r(\mu \cdot t) \left[\frac{\text{Sinh}\, \mu(1-t)}{\text{Sinh}\, \mu}\right]^{w-1} dt \tag{7}$$

where $r = 1, 2, \ldots, w$

and the functions $K_{r(\tau)}$ have the form

$$K_{r(\tau)} = \sum_{k=r}^{w} \frac{(-1)^{k-r}}{k} \begin{pmatrix} k-1 \\ r-1 \end{pmatrix} \left({}^{w}\!/_{k}\, \mathrm{Sinh} \right) \left({}^{\tau}\!/_{k} \right) \tag{8}$$

Here

$$\begin{pmatrix} k-1 \\ r-1 \end{pmatrix} = \frac{(k-1)!}{(r-1)!\ (k-r)!} \tag{9}$$

is the binomial coefficient and

$$\left({}^{w}\!/_{k}\, \mathrm{Sinh} \right) \left({}^{\tau}\!/_{k} \right) = \prod_{i=1}^{k} \frac{\mathrm{Sinh}\left(\frac{w-k+i}{k} \cdot \tau\right)}{\mathrm{Sinh}\left(\frac{i}{k} \cdot \tau\right)} \tag{10}$$

Calculations based on formulas 7-10, made using an electronic computer, allowed us to calculate the relative lengths of the segments (which were expressed in normalized units N/w) in the range of w values from 5 to 30 and the range of μ values from 0 to 60 (with step size 0.1 in the interval $0 \leq \mu \leq 6$, step size 0.5 in the interval $6 < \mu \leq 12$, step size 1.0 in the interval $12 < \mu \leq 20$ and step size 5.0 in the interval $20 < \mu \leq 60$. For the value $\mu = 60$, on the average, the largest segment represents 98.5% of the length of the broken rod and it is independent of the value of w. For the value $\mu = 0$ (special case of B.R. model), for w = 5, the largest segment represents 45.5% of the length of the rod, for w = 10, 29% of the length of the rod, and finally for w = 30, only 13.3% of the length of the rod. The ratios of the population sizes of the crowding species n_1 to N are in the same proportions.

Tables 1-3 give (as an example) the relative population sizes for w = 5, w = 10, and w = 30, with step size $\mu = 1.0$ and $\mu = 5.0$ in the range of μ values from 0 to 60.

RELATIVE ABUNDANCE OF COMPONENT POPULATIONS MAKING UP PHYTOPLANKTON

Component plant populations making up the phytoplankton are mainly represented by sympatric species. Consequently, in rough approximation, all individuals in an association of this type can be considered as physiologically inseparable units (Federov, 1970). The seasonal variations in density are directly correlated with a change in feeding and illuminance conditions. The alteration of dominant forms or even groups of algae in space and time, and the obvious opportunism of the overwhelming number of species (for which the integral in equation 2 is greater than the logarithm of the original number of individuals) allows us to attribute to this trophic group of organisms a relatively high degree of unbalance. Therefore one would expect that a generalized model entailing an additional independent variable (popualtion density) will make it possible to better predict the distribution of

TABLE 1

μ	$\hat{n}_r$				
	1	2	3	4	5
0.0	2.28	1.28	0.78	0.45	0.20
1.0	2.51	1.23	0.70	0.39	0.17
2.0	2.98	1.08	0.54	0.28	0.12
3.0	3.41	0.90	0.41	0.20	0.08
4.0	3.74	0.73	0.31	0.15	0.06
5.0	3.97	0.61	0.25	0.12	0.05
6.0	4.14	0.51	0.21	0.10	0.04
7.0	4.26	0.44	0.18	0.09	0.04
8.0	4.35	0.39	0.16	0.08	0.03
9.0	4.42	0.34	0.14	0.07	0.03
10.0	4.48	0.31	0.13	0.06	0.03
11.0	4.53	0.28	0.11	0.06	0.02
12.0	4.57	0.26	0.10	0.05	0.02
13.0	4.60	0.24	0.10	0.05	0.02
14.0	4.63	0.22	0.09	0.04	0.02
15.0	4.65	0.21	0.08	0.04	0.02
16.0	4.67	0.19	0.08	0.04	0.02
17.0	4.69	0.18	0.07	0.04	0.01
18.0	4.71	0.17	0.07	0.03	0.01
19.0	4.73	0.16	0.07	0.03	0.01
20.0	4.74	0.15	0.06	0.03	0.01
25.0	4.79	0.12	0.05	0.02	0.01
30.0	4.83	0.10	0.04	0.02	0.01
35.0	4.86	0.09	0.03	0.02	0.01
40.0	4.88	0.07	0.03	0.01	0.01
45.0	4.89	0.06	0.03	0.01	0.01
50.0	4.91	0.06	0.02	0.01	0.00
55.0	4.92	0.05	0.02	0.01	0.00
60.0	4.92	0.05	0.02	0.01	0.00

TABLE 2

μ	$\hat{n}_r$									
	1	2	3	4	5	6	7	8	9	10
0.0	2.93	1.93	1.43	1.10	0.85	0.65	0.48	0.34	0.21	0.10
1.0	3.46	1.97	1.35	0.99	0.74	0.55	0.40	0.28	0.17	0.08
2.0	4.61	1.93	1.14	0.76	0.54	0.39	0.28	0.19	0.11	0.05
3.0	5.72	1.72	0.89	0.56	0.39	0.28	0.19	0.13	0.08	0.04
4.0	6.59	1.45	0.69	0.43	0.30	0.21	0.15	0.10	0.06	0.03
5.0	7.20	1.22	0.56	0.35	0.24	0.17	0.12	0.08	0.05	0.02
6.0	7.65	1.03	0.47	0.29	0.20	0.14	0.10	0.07	0.04	0.02
7.0	7.98	0.89	0.40	0.25	0.17	0.12	0.08	0.06	0.03	0.02
8.0	8.23	0.78	0.35	0.22	0.15	0.11	0.07	0.05	0.03	0.01
9.0	8.43	0.70	0.31	0.19	0.13	0.09	0.07	0.04	0.03	0.01
10.0	8.59	0.63	0.28	0.17	0.12	0.08	0.06	0.04	0.02	0.01
11.0	8.72	0.57	0.25	0.16	0.11	0.08	0.05	0.04	0.02	0.01
12.0	8.83	0.52	0.23	0.14	0.10	0.07	0.05	0.03	0.02	0.01
13.0	8.92	0.48	0.21	0.13	0.09	0.06	0.05	0.03	0.02	0.01
14.0	9.00	0.44	0.20	0.12	0.08	0.06	0.04	0.03	0.02	0.01
15.0	9.08	0.41	0.18	0.11	0.08	0.05	0.04	0.03	0.02	0.01
16.0	9.14	0.38	0.17	0.10	0.07	0.05	0.04	0.02	0.01	0.01
17.0	9.20	0.36	0.16	0.10	0.07	0.05	0.03	0.02	0.01	0.01
18.0	9.25	0.34	0.15	0.09	0.06	0.04	0.03	0.02	0.01	0.01
19.0	9.29	0.32	0.14	0.09	0.06	0.04	0.03	0.02	0.01	0.01
20.0	9.33	0.30	0.13	0.08	0.05	0.04	0.03	0.02	0.01	0.01
25.0	9.49	0.24	0.10	0.06	0.04	0.03	0.02	0.01	0.01	0.00
30.0	9.60	0.19	0.08	0.05	0.03	0.02	0.01	0.01	0.01	0.00
35.0	9.67	0.16	0.07	0.04	0.02	0.02	0.01	0.01	0.00	0.00
40.0	9.73	0.14	0.06	0.03	0.02	0.01	0.01	0.01	0.00	0.00
45.0	9.77	0.12	0.05	0.03	0.02	0.01	0.01	0.00	0.00	0.00
50.0	9.80	0.11	0.04	0.02	0.01	0.01	0.00	0.00	0.00	0.00
55.0	9.83	0.10	0.04	0.02	0.01	0.01	0.00	0.00	0.00	0.00
60.0	9.85	0.09	0.03	0.02	0.01	0.00	0.00	0.00	0.00	0.00

TABLE 3

μ	$\hat{n}_r$															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.0	3.99	2.99	2.49	2.16	1.91	1.71	1.54	1.40	1.23	1.17	1.07	0.98	0.89	0.81	0.74	0.68
1.0	5.35	3.50	2.70	2.22	1.89	1.64	1.45	1.28	1.15	1.03	0.94	0.84	0.77	0.69	0.66	0.58
2.0	8.61	4.32	2.85	2.10	1.66	1.36	1.15	0.98	0.87	0.75	0.68	0.59	0.54	0.48	0.43	0.38
3.0	12.43	4.51	2.53	1.71	1.28	1.02	0.85	0.71	0.63	0.53	0.49	0.42	0.38	0.33	0.33	0.27
4.0	15.91	4.10	2.05	1.33	0.98	0.77	0.64	0.54	0.46	0.41	0.36	0.32	0.28	0.25	0.22	0.20
5.0	18.58	3.54	1.66	1.06	0.77	0.61	0.50	0.42	0.36	0.32	0.28	0.25	0.22	0.20	0.18	0.16
6.0	20.57	3.02	1.37	0.87	0.63	0.50	0.41	0.34	0.29	0.26	0.23	0.20	0.18	0.16	0.14	0.08
7.0	22.07	2.60	1.16	0.73	0.53	0.41	0.34	0.28	0.24	0.21	0.18	0.16	0.14	0.13	0.11	0.10
8.0	23.22	2.27	1.00	0.63	0.45	0.35	0.29	0.24	0.20	0.18	0.15	0.14	0.12	0.11	0.09	0.08
9.0	24.12	2.01	0.88	0.55	0.39	0.30	0.25	0.20	0.17	0.15	0.13	0.11	0.10	0.09	0.08	0.07
10.0	24.84	1.80	0.78	0.48	0.34	0.27	0.21	0.18	0.15	0.13	0.11	0.10	0.09	0.08	0.07	0.06
11.0	25.42	1.63	0.70	0.43	0.31	0.23	0.19	0.15	0.13	0.11	0.10	0.08	0.07	0.06	0.06	0.05
12.0	25.90	1.49	0.64	0.39	0.27	0.21	0.17	0.14	0.11	0.10	0.08	0.07	0.06	0.05	0.05	0.04
13.0	26.30	1.37	0.58	0.35	0.25	0.19	0.15	0.12	0.10	0.09	0.07	0.06	0.05	0.05	0.04	0.04
14.0	26.64	1.27	0.54	0.32	0.23	0.17	0.13	0.11	0.09	0.08	0.06	0.05	0.05	0.04	0.03	0.03
15.0	26.93	1.18	0.50	0.30	0.21	0.16	0.12	0.10	0.08	0.07	0.06	0.05	0.04	0.04	0.03	0.03
16.0	27.18	1.11	0.46	0.28	0.19	0.14	0.11	0.09	0.07	0.06	0.05	0.04	0.04	0.03	0.03	0.02
17.0	27.39	1.04	0.43	0.26	0.18	0.13	0.10	0.08	0.07	0.05	0.04	0.04	0.03	0.03	0.02	0.02
18.0	27.58	0.98	0.41	0.24	0.16	0.12	0.09	0.07	0.06	0.05	0.04	0.03	0.03	0.02	0.02	0.02
19.0	27.75	0.93	0.38	0.23	0.15	0.11	0.09	0.07	0.05	0.04	0.04	0.03	0.02	0.02	0.02	0.01
20.0	27.90	0.88	0.36	0.21	0.14	0.10	0.08	0.06	0.05	0.04	0.03	0.03	0.02	0.02	0.01	0.01
25.0	28.44	0.69	0.28	0.16	0.11	0.07	0.05	0.04	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.01
30.0	28.78	0.57	0.23	0.13	0.08	0.06	0.04	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00
35.0	29.01	0.48	0.19	0.10	0.07	0.04	0.03	0.02	0.01	0.01	0.01	0.01	0.00	0/00	0.00	0.00
40.0	29.18	0.41	0.16	0.09	0.05	0.03	0.02	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00
45.0	29.30	0.36	0.14	0.07	0.04	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50.0	29.40	0.32	0.12	0.06	0.04	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
55.0	29.48	0.29	0.11	0.05	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60.0	29.54	0.26	0.10	0.05	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 3 (continued)

μ	$\hat{n}_r$ 17	18	19	20	21	22	23	24	25	26	27	28	29	30
0.0	0.61	0.56	0.50	0.45	0.40	0.35	0.30	0.26	0.22	0.18	0.14	0.10	0.07	0.03
1.0	0.51	0.43	0.36	0.32	0.28	0.24	0.21	0.17	0.14	0.11	0.08	0.05	0.03	0.03
2.0	0.34	0.31	0.27	0.24	0.21	0.18	0.16	0.14	0.11	0.09	0.07	0.05	0.03	0.02
3.0	0.24	0.21	0.19	0.17	0.15	0.13	0.11	0.09	0.08	0.06	0.05	0.04	0.02	0.01
4.0	0.18	0.16	0.14	0.13	0.11	0.10	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01
5.0	0.14	0.13	0.11	0.10	0.09	0.08	0.06	0.06	0.05	0.04	0.03	0.02	0.01	0.01
6.0	0.11	0.10	0.09	0.08	0.07	0.06	0.05	0.04	0.04	0.03	0.02	0.02	0.01	0.01
7.0	0.09	0.08	0.07	0.06	0.06	0.05	0.04	0.04	0.03	0.02	0.02	0.01	0.01	0.00
8.0	0.07	0.07	0.06	0.05	0.05	0.04	0.03	0.03	0.02	0.02	0.01	0.01	0.01	0.00
9.0	0.06	0.05	0.05	0.04	0.04	0.03	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.00
10.0	0.05	0.05	0.04	0.03	0.03	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.00	0.00
11.0	0.04	0.04	0.03	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00
12.0	0.04	0.03	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00
13.0	0.03	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
14.0	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00
15.0	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00
16.0	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
17.0	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18.0	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19.0	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20.0	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25.0	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
55.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

individuals in the natural phytoplankton by species by means of an appropriate selection of the parameter μ. To do this, it is necessary to relate the experimental N values to tabular μ values. In the first place, to make a sufficiently accurate selection of μ, we must know the accuracy with which w and N are being determined in samples of the natural plankton.

The following relationship was determined between the number of species detected and the number of surveyed individuals on the basis of data obtained from a quantitative treatement of two series of samples (each series consisted of 50 samples taken simultaneously) of diatomaceous plankton of different density (thousands and tens of thousands of cells per liter) (Kol'tsova et al., 1971):

$$w = a \log \frac{N}{10} \tag{11}$$

Using these data, I established that by counting approximately 3000 individuals, w can be determined with an error that is less than 20%. Fixing the sample size (by counting the number of plankton counting cells), and counting approximately 3000 cells, also allows the determination of the number of individuals per liter with an error which is less than 20%. This problem will be discussed in greater detail in a paper dedicated to methods used in the study of phytoplankton (Fedorov, 1977). If for some reason(s) the researcher is forced to restrict himself to a sample consisting of a smaller number of individuals, the number of species can be determined by means of calculation. To do this, it suffices to determine the value of the slope a in equation (11) from the data in this nonrepresentative sample, plot the graph of w as a function of log N/10* and then calculate $\hat{w}$ for 3000 individuals. In this case agreement must be sought between the prediction made by the model for the number of individuals N that was found and the calculated value $\hat{w}$.

Therefore, it is clear that for the accuracy with which N and μ were determined, while seeking relations between N and μ, it makes no sense to focus the search on segments whose sizes correspond to species represented by a small number of individuals. It suffices entirely to determine the correspondence between the abundance of the first 5 most prevalent species in the sequence of populations ranked by size ($n_1, n_2, \ldots, n_5$), and the corresponding tabulated values of the lengths of segments. In this case, in Tables 2 and 3, for 10 and 30 species respectively, only 5 columns are left in each table. This simplifies the compilation of a general table for natural plankton. The magnitude of the error with which w is determined allows, in turn, a reduction (in a valid manner) of the number of tables by at least a factor of 2 through the inclusion of only even or odd w values in the general table (for example, $w = 6, 8, \ldots, 30$). In the case when the number of species w found or calculated "does not have" its own table, the table for the value w+1 can be used, since we can assume that in all probability some species was not detected (the case that one species too many was detected is excluded), so that we can correct the error by increasing the sample size. The known population size measurement error allows ascertaining the error with which the parameter μ is determined, which in turn permits us to increase the "step size" used in the compiled summary table. Thus, in the range of μ values from 6 to 20, 1.0 is an acceptable step size, while for $\mu > 20$, this step size can be increased to 5.0. A summary table for phytoplankton is presented below. Using this table, one can find the relationship between the parameter μ and the quantities w and N.

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*Usually the slope varies considerably. Thus, for White Sea diatomaceous plankton, its value varies in the range 8-10. For Arctic plankton (Kara Sea), "a" varies in the range 20-30.

To illustrate how this relationship is found, I present as an example the data used in a quantitative treatment of samples collected in 38 White Sea water area stations (June 1972). The samples were taken at 4 levels (0.5, 2.5, 5.0 and 10.0 m). Samples from the surface level were used to find the relationship, and from the remaining levels only those samples for which log N was less than 4 and greater than 6. The spatial heterogeneity in the distribution of phytoplankton is related to the presence of patches forming microscopic algae concentrations in the photic zone. According to Platt et al. (Platt, Dickie, and Trites, 1970), the diameter of such patches varies in the range from 1.3 to 3.9 km. Using correlation analysis, it was possible to determine for White Sea diatomaceous plankton a relationship between μ and log N and also between μ and (log N, w) [sic]:

$$\mu = 2.1\,(\log N)^2 - 9.6\,\log N - 0.025 \tag{12}$$

$$\mu = 11.1\,(\ln N)^2 - 22.1\,\ln N - 0.025$$

$$\mu = 5.9\,(\log N)^2 - 26.6\,(\log N - \log w) + 44.3\,(\log w)^2 - 15.4\,\log N + 0.9\,\log w + 33.8 \tag{13}$$

where w and N are the number of species and the total number of individuals in one liter of the sample, respectively. Of course, no "mysterious" significance must be attributed to equations (12) and (13). By validating theoretically the existence of a relationship between the associated parameters, they simply enable us to rewrite equation (7) in terms of the quantities whose values were recorded. In practice, they make it possible to find μ on the basis of data about w and N, and to predict the distribution of population sizes for the first five most prevalent species in the sequence of populations ranked by size on the basis of Table 4 for the number of species w (or w+1). The following statement can be made with 100% confidence. The actual concrete form of the relationship between the associated parameters will vary from basin to basin and possibly from season to season (for each basin). Moreover, serious reasons exist which in principle preclude this relationship from being good, i.e., more or less rigorous, definite and invariant. The only statement that can be made with confidence is that the new model will predict the distribution of N individuals among w species better than MacArthur's B.R. model. Therefore, it is hoped that the new model represents progress in the quest for some hypothetically ideal predictive model. It must be assumed that at the present time we only began this quest. This is borne out convincingly by a number of common and special reasons (pertaining in particular to phytoplankton) which are responsible for the discrepancy between the forecast made by the model and reality.

1. Notwithstanding the fact that the introduction of the parameter μ

TABLE 4

w	5					7					9				
μ \ $\hat{n}_r$	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0.0	2.28	1.28	0.78	0.45	0.20	2.59	1.59	1.09	0.76	0.51	2.83	1.83	1.33	1.09	0.75
1.0	2.51	1.23	0.70	0.39	0.17	2.95	1.57	1.00	0.67	0.44	3.31	1.84	1.25	0.89	0.65
2.0	2.93	1.03	0.54	0.28	0.12	3.69	1.44	0.80	0.50	0.31	4.32	1.77	1.03	0.68	0.47
3.0	3.41	0.90	0.41	0.20	0.08	4.40	1.24	0.61	0.36	0.22	5.30	1.56	0.80	0.50	0.34
4.0	3.74	0.73	0.31	0.15	0.06	4.93	1.03	0.47	0.27	0.17	6.05	1.31	0.62	0.38	0.25
5.0	3.97	0.61	0.25	0.12	0.05	5.31	0.85	0.32	0.22	0.13	6.58	1.10	0.50	0.30	0.20
6.0	4.14	0.51	0.21	0.10	0.04	5.58	0.72	0.32	0.18	0.11	6.97	0.93	0.42	0.25	0.17
7.0	4.26	0.44	0.18	0.09	0.04	5.78	0.62	0.27	0.16	0.10	7.25	0.80	0.36	0.22	0.15
8.0	4.35	0.39	0.16	0.08	0.03	5.93	0.55	0.24	0.14	0.08	7.47	0.70	0.31	0.19	0.13
9.0	4.42	0.34	0.14	0.07	0.03	6.05	0.49	0.21	0.12	0.07	7.64	0.63	0.28	0.17	0.11
10.0	4.48	0.31	0.13	0.05	0.03	6.14	0.44	0.19	0.11	0.07	7.77	0.56	0.25	0.15	0.10
11.0	4.53	0.28	0.11	0.06	0.02	6.22	0.40	0.17	0.10	0.06	7.89	0.51	0.23	0.14	0.09
12.0	4.57	0.26	0.10	0.05	0.02	6.28	0.36	0.15	0.09	0.06	7.98	0.47	0.21	0.13	0.09
13.0	4.60	0.24	0.10	0.05	0.02	6.34	0.34	0.15	0.08	0.05	8.06	0.43	0.19	0.12	0.08
14.0	4.63	0.22	0.09	0.04	0.02	6.39	0.31	0.14	0.08	0.05	8.13	0.40	0.18	0.11	0.07
15.0	4.65	0.21	0.08	0.04	0.02	6.43	0.29	0.13	0.07	0.04	8.19	0.37	0.16	0.10	0.07
16.0	4.67	0.19	0.08	0.04	0.02	6.45	0.27	0.12	0.07	0.04	8.25	0.35	0.15	0.09	0.06
17.0	4.69	0.18	0.07	0.04	0.01	6.50	0.26	0.11	0.06	0.04	8.30	0.33	0.14	0.09	0.06
18.0	4.71	0.17	0.07	0.03	0.01	6.53	0.24	0.10	0.05	0.04	8.34	0.31	0.13	0.08	0.05
19.0	4.73	0.15	0.07	0.03	0.01	6.55	0.23	0.10	0.06	0.03	8.38	0.29	0.13	0.08	0.05
20.0	4.74	0.15	0.05	0.03	0.01	6.58	0.22	0.09	0.05	0.03	8.41	0.27	0.12	0.07	0.05
25.0	4.79	0.12	0.05	0.02	0.01	6.67	0.17	0.07	0.04	0.03	8.55	0.21	0.09	0.06	0.04
30.0	4.83	0.10	0.04	0.02	0.01	6.73	0.14	0.06	0.03	0.02	8.64	0.17	0.07	0.04	0.03
35.0	4.86	0.09	0.03	0.02	0.01	6.78	0.12	0.05	0.03	0.02	8.71	0.15	0.06	0.03	0.02
40.0	4.88	0.07	0.03	0.01	0.01	6.21	0.10	0.04	0.02	0.01	8.75	0.13	0.05	0.03	0.02
45.0	4.89	0.06	0.03	0.01	0.01	6.84	0.09	0.04	0.02	0.01	8.79	0.11	0.04	0.02	0.01
50.0	4.91	0.06	0.02	0.01	0.00	6.86	0.08	0.03	0.02	0.01	8.82	0.10	0.04	0.02	0.01
55.0	4.92	0.05	0.02	0.01	0.00	6.88	0.07	0.03	0.01	0.01	8.84	0.09	0.03	0.02	0.01
60.9	4.92	0.05	0.02	0.01	0.00	6.89	0.06	0.02	0.01	0.01	8.86	0.08	0.03	0.01	0.01

TABLE 4 (continued)

w	11					13					15				
μ \ $\hat{n}_r$	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0.0	3.02	2.02	1.52	1.19	0.94	3.18	2.10	1.68	1.35	1.10	3.32	2.32	1.82	1.43	1.23
1.0	3.61	2.08	1.45	1.08	0.83	3.83	2.29	1.64	1.25	0.98	4.11	2.42	1.80	1.40	1.12
2.0	4.88	2.08	1.24	0.85	0.61	5.40	2.36	1.44	1.00	0.74	5.27	2.63	1.63	1.15	0.87
3.0	6.14	1.87	0.98	0.63	0.44	6.93	2.18	1.15	0.75	0.54	7.68	2.47	1.32	0.87	0.63
4.0	7.11	1.59	0.76	0.48	0.33	8.14	1.87	0.90	0.58	0.41	9.12	2.15	1.04	0.67	0.48
5.0	7.82	1.34	0.62	0.39	0.27	9.04	1.58	0.73	0.49	0.33	10.18	1.82	0.85	0.54	0.39
6.0	8.32	1.14	0.51	0.32	0.22	9.65	1.34	0.61	0.39	0.27	10.95	1.55	0.71	0.45	0.32
7.0	8.70	0.98	0.44	0.28	0.19	10.42	1.16	0.52	0.33	0.24	11.53	1.33	0.60	0.38	0.28
8.0	8.98	0.86	0.39	0.24	0.17	10.40	1.01	0.45	0.29	0.21	11.97	1.17	0.52	0.33	0.24
9.0	9.21	0.76	0.34	0.21	0.15	10.77	0.90	0.40	0.25	0.15	12.33	1.03	0.46	0.29	0.21
10.0	9.39	0.69	0.31	0.19	0.13	11.00	0.81	0.36	0.23	0.15	12.61	0.93	0.41	0.26	0.19
11.0	9.54	0.62	0.28	0.17	0.12	11.23	0.73	0.33	0.21	0.15	12.85	0.84	0.37	0.23	0.17
12.0	9.67	0.57	0.25	0.16	0.11	11.36	0.67	0.30	0.19	0.13	13.05	0.76	0.34	0.21	0.15
13.0	9.78	0.52	0.23	0.14	0.10	11.56	0.61	0.27	0.17	0.12	13.22	0.70	0.31	0.19	0.14
14.0	9.88	0.48	0.21	0.13	0.09	11.62	0.57	0.25	0.15	0.11	13.37	0.65	0.28	0.18	0.13
15.0	9.96	0.45	0.20	0.12	0.09	11.75	0.53	0.23	0.14	0.10	13.50	0.60	0.25	0.16	0.12
16.0	10.03	0.42	0.18	0.11	0.08	11.82	0.49	0.21	0.13	0.09	13.51	0.56	0.24	0.15	0.11
17.0	10.10	0.39	0.17	0.11	0.07	11.90	0.46	0.20	0.12	0.09	13.71	0.52	0.23	0.14	0.10
18.0	10.15	0.37	0.16	0.10	0.07	11.96	0.43	0.19	0.12	0.08	13.80	0.49	0.21	0.13	0.09
19.0	10.21	0.35	0.15	0.09	0.06	12.04	0.41	0.18	0.11	0.08	13.88	0.46	0.20	0.12	0.08
20.0	10.25	0.33	0.14	0.09	0.06	12.16	0.38	0.15	0.10	0.07	13.95	0.44	0.19	0.11	0.08
25.0	10.43	0.26	0.11	0.07	0.04	12.32	0.30	0.16	0.08	0.05	14.22	0.34	0.14	0.08	0.05
30.0	10.55	0.21	0.09	0.05	0.03	12.47	0.25	0.10	0.06	0.04	14.39	0.28	0.11	0.07	0.04
35.0	10.64	0.18	0.07	0.04	0.03	12.52	0.21	0.08	0.05	0.03	14.51	0.24	0.09	0.05	0.03
40.0	10.70	0.15	0.06	0.03	0.02	12.54	0.18	0.07	0.04	0.02	14.59	0.21	0.08	0.04	0.03
45.0	10.74	0.13	0.05	0.03	0.02	12.71	0.15	0.06	0.03	0.02	14.65	0.18	0.07	0.04	0.02
50.0	10.78	0.12	0.04	0.02	0.01	12.74	0.14	0.05	0.03	0.02	14.70	0.16	0.06	0.03	0.02
55.0	10.81	0.11	0.04	0.02	0.01	12.77	0.12	0.05	0.02	0.01	14.74	0.14	0.05	0.03	0.01
60.0	10.83	0.10	0.03	0.02	0.01	12.81	0.11	0.04	0.02	0.01	14.77	0.13	0.05	0.02	0.01

TABLE 4 (continued)

w	17					19					21				
μ \ $\hat{n}_r$	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0.0	3.44	2.44	1.94	1.61	1.36	3.55	2.55	2.05	1.71	1.46	3.65	2.69	2.45	1.81	1.86
1.0	4.33	2.65	1.95	1.53	1.25	4.52	2.80	2.09	1.6	1.37	4.70	2.96	2.21	1.73	1.78
2.0	6.31	2.89	1.81	1.29	0.98	6.72	3.13	1.98	1.42	1.09	7.11	3.37	2.15	1.65	1.71
3.0	8.40	2.76	1.49	0.99	0.73	9.08	3.05	1.65	1.10	0.82	9.74	3.35	1.82	1.22	0.63
4.0	10.08	2.42	1.18	0.76	0.55	11.01	2.69	1.32	0.85	0.62	11.95	2.95	1.46	0.94	0.59
5.0	11.33	2.05	0.96	0.61	0.44	12.46	2.29	1.07	0.68	0.50	15.57	2.52	1.15	0.78	0.53
6.0	12.24	1.75	0.80	0.51	0.37	13.52	1.95	0.89	0.57	0.41	14.80	2.15	0.98	0.62	0.47
7.0	12.93	1.51	0.68	0.43	0.31	14.33	1.68	0.76	0.48	0.35	15.72	1.85	0.83	0.59	0.39
8.0	13.46	1.32	0.59	0.38	0.27	14.95	1.46	0.66	0.42	0.30	16.44	1.61	0.72	0.46	0.31
9.0	13.88	1.17	0.52	0.33	0.24	15.44	1.30	0.58	0.37	0.25	17.01	1.43	0.63	0.40	0.28
10.0	14.23	1.04	0.46	0.29	0.21	15.85	1.16	0.51	0.32	0.23	17.47	1.28	0.55	0.35	0.23
11.0	14.51	0.94	0.42	0.26	0.19	16.18	1.05	0.46	0.29	0.21	17.85	1.15	0.56	0.32	0.21
12.0	14.75	0.86	0.38	0.24	0.17	16.46	0.96	0.42	0.26	0.19	18.16	1.05	0.48	0.28	0.20
13.0	14.95	0.79	0.35	0.22	0.15	16.69	0.88	0.38	0.24	0.17	18.43	0.97	0.42	0.25	0.18
14.0	15.13	0.73	0.32	0.20	0.14	16.89	0.81	0.35	0.22	0.15	18.66	0.89	0.38	0.24	0.17
15.0	15.28	0.68	0.29	0.18	0.13	17.07	0.75	0.32	0.20	0.14	18.86	0.89	0.35	0.22	0.15
16.0	15.41	0.63	0.27	0.17	0.12	17.22	0.70	0.30	0.18	0.13	19.03	0.77	0.33	0.20	0.14
17.0	15.53	0.59	0.25	0.15	0.11	17.35	0.68	0.28	0.17	0.12	19.18	0.75	0.31	0.19	0.13
18.0	15.63	0.56	0.24	0.14	0.10	17.47	0.52	0.26	0.15	0.11	19.31	0.68	0.29	0.17	0.12
19.0	15.73	0.52	0.22	0.13	0.09	17.57	0.58	0.25	0.15	0.10	19.42	0.65	0.27	0.16	0.11
20.0	15.81	0.50	0.21	0.13	0.09	17.67	0.55	0.23	0.14	0.10	19.53	0.81	0.25	0.15	0.10
25.0	16.11	0.39	0.16	0.09	0.09	18.81	0.44	0.18	0.10	0.07	19.91	0.48	0.20	0.11	0.08
30.0	16.31	0.32	0.13	0.07	0.05	18.23	0.36	0.14	0.08	0.05	20.15	0.40	0.15	0.09	0.06
35.0	16.44	0.27	0.11	0.06	0.04	18.38	0.30	0.12	0.07	0.04	20.31	0.34	0.13	0.07	0.04
40.0	16.54	0.23	0.09	0.05	0.03	18.48	0.26	0.10	0.05	0.03	20.43	0.29	0.11	0.08	0.04
45.0	16.61	0.21	0.08	0.04	0.02	18.56	0.23	0.09	0.05	0.03	20.51	0.25	0.10	0.05	0.03
50.0	16.66	0.18	0.07	0.04	0.02	18.62	0.20	0.08	0.04	0.02	20.58	0.23	0.02	0.04	0.01
55.0	16.70	0.16	0.06	0.03	0.02	18.67	0.18	0.07	0.03	0.02	20.63	0.20	0.07	0.04	0.01
60.0	16.74	0.15	0.05	0.03	0.01	18.71	0.17	0.06	0.03	0.02	20.68	0.18	0.07	0.03	0.01

TABLE 4 (continued)

w	23					25					27				
μ \ $\hat{n}_r$	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0.0	3.73	2.73	2.23	1.90	1.65	3.82	2.82	2.32	1.98	1.73	3.89	2.29	2.39	2.05	1.81
1.0	4.86	3.08	2.33	1.28	1.53	5.02	3.21	2.44	1.99	1.67	5.16	3.33	2.55	2.03	1.76
2.0	7.47	3.59	2.31	1.68	1.31	7.82	3.81	2.47	1.81	1.41	8.14	4.02	2.62	1.93	1.51
3.0	10.37	3.60	1.98	1.33	0.99	10.99	3.86	2.14	1.44	1.08	11.59	4.12	2.50	1.55	1.16
4.0	12.82	3.21	1.59	1.03	0.76	15.71	3.47	1.72	1.12	0.82	14.59	3.73	1.86	1.20	0.89
5.0	14.89	2.75	1.29	0.82	0.60	15.80	2.98	1.39	0.29	0.65	16.91	3.20	1.50	0.96	0.70
6.0	16.08	2.34	1.07	0.69	0.50	17.35	2.54	1.15	0.73	0.54	18.64	2.73	1.24	0.79	0.58
7.0	17.13	2.02	0.90	0.57	0.42	18.53	2.18	0.98	0.62	0.45	19.94	2.35	1.04	0.66	0.48
8.0	17.94	1.75	0.78	0.49	0.36	19.44	1.91	0.84	0.53	0.39	20.95	2.05	0.91	0.57	0.41
9.0	18.58	1.56	0.69	0.43	0.31	20.16	1.69	0.74	0.47	0.34	21.74	1.82	0.80	0.50	0.36
10.0	19.10	1.39	0.61	0.38	0.28	20.73	1.51	0.66	0.41	0.30	22.37	1.63	0.71	0.44	0.32
11.0	19.52	1.26	0.55	0.34	0.24	21.21	1.37	0.59	0.37	0.26	22.89	1.47	0.64	0.39	0.28
12.0	19.88	1.15	0.50	0.31	0.22	21.60	1.25	0.54	0.33	0.24	23.32	1.34	0.58	0.35	0.25
13.0	20.18	1.06	0.45	0.28	0.20	21.92	1.15	0.49	0.30	0.21	23.67	1.24	0.53	0.32	0.23
14.0	20.43	0.98	0.42	0.26	0.18	22.20	1.06	0.45	0.27	0.19	23.98	1.14	0.49	0.29	0.21
15.0	20.65	0.91	0.39	0.23	0.16	22.44	0.99	0.42	0.25	0.18	24.23	0.06	0.45	0.27	0.19
16.0	20.84	0.85	0.36	0.22	0.15	22.65	0.92	0.39	0.23	0.16	24.46	1.00	0.42	0.25	0.17
17.0	21.00	0.79	0.33	0.20	0.14	22.83	0.86	0.36	0.22	0.15	24.65	0.93	0.39	0.23	0.16
18.0	21.14	0.75	0.31	0.19	0.13	22.93	0.81	0.34	0.20	0.14	24.82	0.88	0.37	0.22	0.15
19.0	21.27	0.71	0.29	0.17	0.12	23.12	0.77	0.32	0.19	0.13	24.97	0.83	0.34	0.20	0.14
20.0	21.39	0.67	0.28	0.16	0.11	23.25	0.76	0.30	0.16	0.12	25.11	0.79	0.32	0.19	0.13
25.0	21.80	0.53	0.21	0.12	0.03	23.70	0.58	0.23	0.13	0.09	25.60	0.62	0.25	0.14	0.10
30.0	22.07	0.44	0.17	0.10	0.06	25.98	0.47	0.19	0.11	0.07	25.90	0.51	0.20	0.11	0.07
35.0	22.24	0.37	0.14	0.08	0.05	24.18	0.40	0.16	0.09	0.05	26.11	0.43	0.17	0.09	0.06
40.0	22.37	0.32	0.12	0.07	0.04	24.32	0.35	0.13	0.07	0.04	26.26	0.37	0.14	0.08	0.05
45.0	22.47	0.28	0.11	0.05	0.03	24.42	0.30	0.11	0.05	0.04	26.37	0.33	0.12	0.07	0.04
50.0	22.54	0.25	0.09	0.05	0.03	24.50	0.27	0.10	0.05	0.03	26.46	0.29	0.11	0.06	0.03
55.0	22.60	0.22	0.08	0.05	0.03	24.56	0.24	0.09	0.04	0.02	26.53	0.26	0.10	0.05	0.03
60.0	22.65	0.20	0.07	0.04	0.02	24.62	0.22	0.08	0.04	0.02	26.59	0.24	0.09	0.04	0.02

TABLE 4 (continued)

μ \ $\hat{n}_r$ \ w	29				
	1	2	3	4	5
0.0	3.96	2.96	2.46	2.43	1.88
1.0	5.29	3.44	2.65	2.18	1.85
2.0	8.45	4.22	2.77	1.95	1.81
3.0	12.19	4.38	2.45	1.66	1.24
4.0	15.47	3.96	1.99	1.29	0.95
5.0	18.02	3.43	1.66	1.09	0.75
6.0	19.93	2.92	1.32	0.84	0.61
7.0	21.36	2.52	1.12	0.71	0.51
8.0	22.46	2.20	0.97	0.61	0.44
9.0	23.33	1.95	0.85	0.53	0.38
10.0	24.02	1.75	0.76	0.47	0.35
11.0	24.58	1.58	0.68	0.42	0.30
12.0	25.04	1.44	0.62	0.38	0.27
13.0	25.42	1.33	0.56	0.34	0.24
14.0	25.75	1.23	0.52	0.31	0.22
15.0	26.05	1.14	0.43	0.29	0.20
16.0	26.27	1.07	0.45	0.27	0.19
17.0	26.48	1.00	0.42	0.25	0.17
18.0	26.65	0.95	0.39	0.23	0.16
19.0	26.82	0.89	0.37	0.22	0.15
20.0	26.97	0.85	0.35	0.20	0.14
25.0	27.49	0.67	0.27	0.16	0.10
30.0	27.82	0.55	0.22	0.12	0.08
35.0	28.05	0.47	0.18	0.10	0.06
40.0	28.21	0.40	0.15	0.08	0.05
45.0	28.33	0.35	0.13	0.07	0.04
50.0	28.42	0.31	0.12	0.06	0.03
55.0	28.50	0.28	0.10	0.05	0.03
60.0	28.55	0.25	0.09	0.04	0.02

makes the model more sophisticated, it is still too simple to reflect the outcome of events in such a dynamic system as phytoplankton in which the presence of homeostasis is determined by a possible rearrangement of the structure (i.e., due to a change in the relative abundance of populations!), and by a suppression of sustained relatively stable functional characteristics (Fedorov, 1974).

2. The application of static models to dynamic systems inevitably encounters difficulties related to the probabilistic description of the variables. MacArthur (1960), who tried to overcome these difficulties, approximated the variations in the population sizes of sympatric species by a straight line and separated the species into species in equilibrium and opportunistic species. He excluded the latter from his model on the (rather unconvincing) grounds that among opportunistic species, "their relative abundance is of little biological interest since it is controlled by variations in the climate and other external factors exerting an effect on the rank r" (ibid.). By this discriminatory act, he eliminated the dependence of the model on N, i.e., he reduced the model to consideration of a special case. In reality even the population sizes of so-called equilibrium species fluctuate, which is an objective reason for the errors made in predictions. Returning to the description of events in a dynamic association, using a static model reflecting the result of the action of regulatory mechanisms operating in the ecosystem, "poor agreement" between the forecasts made by the model and nature should be anticipated due to this reason alone: a number of "wild" results (since a probabilistic model utilizes average values, thus allowing for a certain degree of scatter of the data about the mean).

Thus, the model is static because it reflects the result of the action of regulatory mechanisms but does not reflect the rates of the processes regulating these mechanisms. The observed result, which manifests itself in a change of population sizes, reflects the effect of external conditions which had a determining effect in the basin somewhat earlier. More accurately, this "earlier" is measured by the lag in "response" (i.e., recorded changes in the structure of the association) to disturbances in the biotype (for phytoplankton usually 2-4 days).

3. The introduction of the parameter μ in the model in implicit form postulates a direct relationship between N and the mechanism of competitive crowding out of species (in more precise terminology the shoving of the rare species into the reserve or "far end" of the ranked sequence). This is undoubtedly a crude assumption, since the fluctuations may be related both to keener competition for food observed in phytoplankton in the summer period (Fedorov, Traskin and Dauda, 1973), and to the beginning of seasonal succession observed in the spring phytoplankton bloom period. In the latter case, the end of phytoplankton "flourishing" is not necessarily due to having exhausted biogenic elements and to keener competition for food; it may also be due to biotic causes, i.e., a sharp increase in the number and activity of phagocytes.

4. An important misgiving which I have not been able to overcome so far is that the longest segment predicted by the exponentially broken rod model is in fact not proportional to the individuals in a single most abundant species, but rather to the individuals in the crowding species. The latter

can be isolated using a method that was described earlier (Fedorov, 1969). Although in the overwhelming number of cases there turns out to be only one crowding species, sometimes there are two, and very rarely three such species. This misgiving applies above all to phytoplankton representing an association of individuals which are nearly indistinguishable. If this misgiving turns out to be correct, after the population sizes of the sympatric species are ordered in decreasing sequence, the dominant species must be isolated statistically, and the predictions made by the E.B.R. model must be compared with the ranked sequence of values n_j in which the first term is

$$n_1 = \sum_{i=1}^{K} n_i$$

and K is the number of isolated dominant species. I cannot state conclusively that the original prerequisites of the model were violated, however, I do attest to the fact that the values of μ calculated on the basis of equations (12) and (13) refer us in the generalized table to a row whose forecasts are in better agreement with $\hat{n}_1$ than n_1.

5. For phytoplankton, the anticipated good agreement with the predictions made by the E.B.R. model should occur if at least two conditions are satisfied: 1) the individuals in the association are inadequately supplied with mineral feed (when Liebig's law comes into force), and 2) presence of more or less homogeneous physiological state of cells (when the proportion of dead individuals is not excessively high). When the phytoplankton densities are high, the proportion of inactive individuals (with yellow and green luminescence in ultraviolet light) can be substantial. Thus, for 17 species of diatomaceous White Sea plankton, in 7 samples taken from the surface level in one day, the individuals with red luminescence in ultraviolet light (i.e., individuals which were undoubtedly alive) represented approximately 67% (for $\overline{N}$ on the order of several tens of thousands of cells/liter). Therefore it would be more correct to compare the ratios of live cells with the predictions made by the model. Since this cannot always be realized technically, the percentage of inactive cells or cells showing little activity can be considered as the main source of errors for high values of N, because under these conditions, it is very unlikely that the individuals have a surplus supply of mineral feed components. For relatively low phytoplankton densities, the proportion of "dead" individuals is relatively low. Therefore, it appears that in this case the relationship between μ and N is not detected because of an adequate supply of mineral feed to individuals. However, low biomasses in the presence of surplus food can be observed only either during sufficiently active consumption of microscopic algae by zooplankton organisms, or in the presence of a definitely unfavorable effect of certain abiotic factors such as light (too much or too little) and low temperature. In the second variant it may happen that some species (for example, a sciophilous and relatively psychrophilic species) which is better adapted to these conditions than the other species can sharply increase its population size, i.e., it displays opportunism. As a result, the observed high value of N and a high value of μ will not reflect the results of the crowding out of partners by this species, and it is not completely clear what kind of effect this case will have on the quantitative aspect of the relation between the parameter μ and the values of N and w.

ADEQUACY OF E.B.R. MODEL

To evaluate objectively the advantages of the MacArthur model and the E.B.R. model, we compared the agreement between the data and their forecasts using an estimate of the variance which was calculated as the mean square difference between the observed population size n_i and the population size $\hat{n}_i$ calculated on the basis of each model:

$$s^2 = \frac{\sum_{i=1}^{w} (n_i - \hat{n}_i)^2}{w} \tag{14}$$

We assumed that a smaller variance indicates a better forecast made by the model. Diatomaceous plankton samples collected near the Karelski coast of the White Sea during the vegetative season at the level below the surface were used for comparison purposes.

To obtain a generalized seasonal index, we used the mean estimated variance averaged over all stations

$$\bar{s}^2 = \frac{\sum_{1}^{K} s^2}{K} \tag{15}$$

where K is the number of stations. A comparison of the variances showed that they differ significantly. Therefore one can assert with a high degree of confidence that better agreement between the observed and calculated distribution of individuals among species is undoubtedly obtained using the E.B.R. model.

Fedorov and Kol'tsova (1972) demonstrated earlier a close relationship between the pattern indicating the decrease in the sequence of populations ranked by size and the density of the phytoplankton. They found that good agreement with the forecasts made by MacArthur's R.C. model was only observed when the density was relatively low (1.10^2-1.10^4 cells/l). To analyze patterns where agreement was poor, we isolated the so-called empirical type (1.10^6-10.10^7 cells/l), which we named after A. Hutchinson (1960), and a certain mixed case for intermediate population sizes. The E.B.R. model greatly simplifies the situation and allows further development of the idea of complementarity as the key principle in the formation of the structure of a phytoplankton association. This principle consists of the following: due to succession and competition for food, which is keenest among ecologically close species, when equilibrium is attained in the ecosystem, ecologically remote species thrive at the same time. This is because ecologically remote species, whose needs appear to be mutually complementary, make "complementary contact," which in turn permits the most efficient utilization of the biotype resources. Ultimately, this leads to a mosaic structure of sympatric species associations as the main characteristic of an organization which reproduces sufficiently faithfully MacArthur's case when the fundamental "niches do not overlap much but there are no free gaps between them" (MacArthur, 1957).

DISCUSSION

Curiously, attempts were made to explain the poor agreement between the ecology and the forecasts made by MacArthur's model by practically anything except the restrictive original assumptions. The most exhaustive "biological analysis" of the broken rod model was made by Ch. King (1964) to whose study we refer the reader interested in the details of the problem. Somewhat later the model was improved by Pilow and Arnoson (1966) and Vandermeyer and MacArthur (1966); however, this did not result in an appreciable improvement of the forecasts made by the model. Apparently the first person to realize the weakness of the original prerequisites in the model was Robert MacArthur himself. At any rate, in 1966, MacArthur (1966) agreed with the remarks made by Pilow and retracted his model in very elegant and restrained words expressing the hope that "it is used only as a rough approximation of the ecology of associations which should be allowed to die a natural death."

However, MacArthur's "renunciation" did not undermine the popularity of the model among ecologists. Kon (1968), Inger (1965), MacDonald (1969), Fedorov (1971), and Fedorov and Kol'tsova (1972) continued to use the model even after Hairston (1969) demonstrated that good agreement between its predictions and the observed abundance could be obtained by an appropriate selection of the sample size. From this Hairston concluded that agreement with the forecasts made by the model does not depend on the ecological characteristics of the system considered and that consequently MacArthur's model is devoid of ecological meaning. In a study titled "Another Look at the Relative Abundance of Species," I analyzed Hairston's arguments, and in my opinion, presented arguments which can cast some doubt on Hairston's discouraging conclusion (Fedorov, to be published). The secret for the tenacity of MacArthur's model is simple. MacArthur constructed a model for which 1) the mathematical calculations are far from trivial, but can be carried out to the end; 2) a probable biological prerequisite is elegantly reduced to the problem of randomly partitioning a segment into parts; and 3) in *some cases* good agreement is observed between the forecasts made by the model and the facts.

The great elegance and genuine beauty of the model consist of these three items which prevent one from attributing the success of its forecasts to pure chance. Beauty and elegance must be a necessary attribute determining the properties of a model. The deep intrinsic soundness of this criterion was realized in practice by Migdal (1976), who wrote: "The concept of beauty plays an important part in checking the validity of results and in finding new laws. It reflects in our consciousness the harmony which exists in nature." A reflection of this harmony reigning in nature was the conviction that the pattern in which the abundance increases in the sequence of populations ranked by size will *always* be a kind of "counterpart" of the relations between partners in one ecological grouping which unifies species with a similar specialization in the association. Therefore, the concept of mechanisms responsible for the prearrangement of the structure of an ecosystem includes *a priori* a natural element "which is hidden in the harmony of parts grasped only by the mind" (Henri Poincare, cited by Migdal, 1976). I would like to illustrate the importance of the natural element in decision making. During a discussion of the specific properties of the E.B.R. model, Valeriy Nikolayevich Tutuballin did not like the effect of the ends of the rod which is essential in the form of the generalized MacArthur distribution proposed by me. Tutuballin was inclined toward the idea that "it was more natural to let the points fall on a circle." In the case of a uniform distribution, when w + 1 points fall independently, in the end it makes no difference whether they fall on a rod or circle. For the case reflecting "the struggle for existence," the position of individual points must be made dependent "so that they can repel each other." Trying to select a formal analogue which simulates reality to which appropriate mathematical tools can be applied, Tutuballin proposes the use of Dayson's model (generally applied to the energy levels of atomic nuclei). Essentially this model seems to simulate successfully the conditions in natural associations. It is presented below as discussed by Tutuballin:

"Let us consider a circle lying in some plane. Suppose that infinitely long and infinitely thin charged filaments stand out perpendicularly to the plant at the points $Q_1,\ldots,Q_n$ on this circle. Then, as is well known, they repel each other and the energy of the whole system is

$$w = \sum_{k<j} \ln \left| e^{iQ_k} - e^{iQ_j} \right| \tag{16}$$

Let us assume a Gibbs distribution for the points $Q_1,\ldots,Q_n$, i.e., a distribution with the density

$$C_{n\beta} \exp\,(-\beta w) \tag{17}$$

where $\beta = 1/T$ and T is the absolute temperature. Then we obtain the problem of the equilibrium distribution of the points $Q_1,\ldots,Q_n$ which are in thermal motion at the temperature T, and repel each other according to the laws of electrostatics. As the temperature approaches infinity, this model becomes a uniform distribution of independent points, i.e., it gives MacArthur's law. At a finite temperature, the parts into which the circle is partitioned are more nearly equal in magnitude than is assumed by MacArthur's law (at zero temperature the parts are equal). "Consequently," Tutuballin concludes, "either everything discussed makes no biological sense, or biological systems must exist, in which due to the struggle for existence, the resulting distribution of the species by population size is more nearly uniform than that obeying MacArthur's law." The last conclusion drawn by Tutuballin is a sufficiently strong argument for asking the question as to which model (among various models) simulates more realistically the situation in an association. It is possible that for some groups of sympatric species in a very mature association (an example of which should be sought in tropical forest associations), the forecasts made by the Dayson-Tutuballin model will come closer to reality than the predictions made by the MacArthur and E.B. R. models. In regard to phytoplankton, it appears that the overwhelming number of observations is indicative of the opposite pattern, a fact which incidentally can be interpreted as an argument favoring the concept of the "immaturity" of plankton associations existing under conditions in which the biotype is disturbed continuously. Besides, forecasts made by models with rather rough approximation hardly force us to accept an alternative in the sense that one model is unequivocally acknowledged as either definitely valid or invalid. If the E.B.R. model appears to be inadequate because of the absence of a direct relationship between the density of the individuals and the limiting factors, at least there is the possibility that the model can be improved by introducing a new variable in it. If ultimately even this cannot be done, I will be consoled by the knowledge that, nonetheless, the E.B.R. model makes predictions which agree better with the facts (at least as far as phytoplankton is concerned) than those made by MacArthur's B.R. model.

CONCLUDING REMARKS

It appears that my interest which focused on finding a more general solution to the problem inspired several enthusiasts interested in general problems of biology. Every one of them contributed some of his work toward carrying out the appropriate calculations, and undoubtedly enhanced the overall progress made in the solution of the more general version of the problem. As was to be expected, the approaches as well as the degree of their comprehension varied considerably. Tutuballin's model is interesting. It includes at least two items out of the three which make the MacArthur model elegant. Should it turn out that it conforms to certain ecological facts, its longevity in ecology can be predicted almost with certainty.

V. A. Svetlosanov's study, dedicated to a solution of the generalized problem, is unfortunately mathematically incorrect. In his study formula (1) is invalid for any function $w(\alpha) \not\equiv \alpha$, and formulas (2) and (3) are invalid no matter what assumptions are made about $w(\alpha)$. However, even if the invalid formulas are simply ignored, things are even more complicated in the ensuing calculations. Thus, Svetlosanov obtains the relative distribution of the species from expressions (6) and (7), defining some decreasing sequence of numbers. At the same time it is not clear how these numbers must be related to the average lengths of the parts obtained or to some probabilities (the latter was done by author in the simplest case when the points were uniformly distributed and the number of points was greater than one). Unfortunately the shortcomings pointed out above make this study less valuable. In fact, its applicability turned out to be too limited and it will therefore gradually "die out."

In conclusion I would like to acknowledge my deep personal gratitude to my colleagues and friends. Among these I thank above all V. N. Tutuballin, M. Ye. Vinogradov, V. V. Nalimov, T. I. Kol'tsova and Yu. Barabasheva for their valuable help during the discussion of my study and also their useful critical comments made during the writing of the manuscript and its preparation for publication.

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IMPACT OF RADIOACTIVITY ON THE MARINE ENVIRONMENT

by

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ABSTRACT

For the past three decades, man has released artificial radionuclides into the marine environment predominantly by global fallout from nuclear weapons testing and by controlled releases from nuclear power and reprocessing plants. A number of ecological and experimental studies have been conducted to determine the effect of these releases on marine organisms. Results of these studies indicate that, within our present state of knowledge, measurable effects on populations or ecosystems have not been observed either on a global or regional scale.

INTRODUCTION

Contamination of the marine environment by radionuclides has caused much concern regarding the possible effects of irradiation on marine species. As a result, considerable research has been conducted over the past two decades in an attempt to determine if the presence of artificial radionuclides in the oceans has affected marine organisms deleteriously. As new information relative to the effects of irradiation on marine organisms becomes available, the possible effect of anthropogenic additions of radionuclides in the marine environment must be constantly re-evaluated.

One problem in accomplishing this task is to define the term *effect* in relation to radiation damage. For purposes of this discussion, we define effect as "the result of an environmental stress or perturbation which causes an alteration in the functioning or form of a biological system at any level of complexity (cellular, organ, individual, population, ecosystem)." Templeton et al. (1976) argue that in the marine environment, populations and ecosystems, not individuals, should be our ultimate concern. If we accept this argument, then radiation damage to cells, organs and individuals is significant only when these effects are manifest at the population or ecosystem level.

Three basic methods are used to assess the importance of anthropogenic additions of radionuclides to natural environments, their resultant dose and their ultimate irradiation effects on marine organisms. One method compares natural background dose rates to aquatic organisms with dose rates received from radionuclides introduced by man. In the second, the dose rate from anthropogenic radionuclides can be compared with results of laboratory irradiation experiments with marine species. Lastly, field studies can be conducted in the particular marine environment receiving artificial radionuclides. The latter attempts to distinguish changes in ecological structure or function (i.e., standing crop, species diversity, productivity, etc.) that can be related to increased levels of radiation. In the following discussion, each method will be examined in an effort to evaluate the impact that man has had on the marine environment by the introduction of artificial radionuclides.

COMPARISON OF NATURAL BACKGROUND DOSE RATES TO MARINE SPECIES WITH THOSE FROM ARTIFICIAL RADIONUCLIDES

The total concentration of natural radionuclides in water, sediments and biota, and their subsequent dose to marine organisms is difficult to assess because of the existence of over 60 radionuclides of natural origin and their variable distribution in the ocean (Folsom and Harley, 1957; Koczy and Rosholt, 1962; Cherry, 1964; Mauchlin and Templeton, 1964; Lal and Peters, 1967; and Joseph et al., 1971). In addition, dose rates to marine organisms from both natural and artificial radionuclides will vary as a function of such variables as position in the water column, proximity to and geological composition of sediments and submerged strata, specificity for adsorption and accumulation, food intake, etc.

In spite of the variables listed above, Woodhead (1973) and Woodhead et al. (1976) compared background dose rates with dose rates from global fallout and from radioactive discharge in two marine environments. These two locations were the portion of the Irish Sea near the nuclear fuel reprocessing plant at Windscale and the Blackwater estuary, England, which receives radioactive effluent from the Bradwell nuclear power station. They summarized available data on environmental concentrations of natural and artificial radionuclides and applied this information to dosimetry models for phytoplankton, zooplankton, mollusks, crustaceans and fish.

Estimates of dose rates from fallout radionuclides are quite variable, but are on the same order as background dose rates (Table 1). Most of the background dose rates for phytoplankton, zooplankton and pelagic fish resulted from incorporated ^{210}Po and ^{40}K. For mollusks, crustaceans and demersal fish, sediments delivered a dose rate similar to that received from incorporated activity. For fallout, the organisms' body burden of artificial radionuclides, particularly ^{137}Cs, contributed the most significant fraction of the dose, although ^{239}Pu in phytoplankton and ^{90}Sr/^{90}Y in zooplankton and crustacea were of some importance.

The releases of radionuclides from Windscale produced significantly higher doses to marine organisms in the immediate vicinity of the discharge than from either fallout or background (Table 1). In addition, 970 fish tagged and recaptured near the point of discharge gave a mean dosimeter reading of 350 μrad hr^{-1} (Pentreath et al., 1973).

In the Blackwater estuary the dose rate from radionuclides released by the power plant could be estimated only for oysters and other benthic organisms and were significantly below background estimates. The total number of curies of radioactivity (excluding tritium) released annually by this nuclear power station exceeds the amount released by both boiling water and pressurized nuclear reactors in the United States by a factor of two to ten (Joseph et al., 1971; Woodhead, 1973).

COMPARISON OF LABORATORY IRRADIATION EXPERIMENTS WITH ENVIRONMENTAL DOSE RATES

In recent years, several review articles have summarized laboratory irradiation experiments on both the lethal and nonlethal responses of marine organisms to radiation (Polikarpov, 1966; Rice and Wolfe, 1971; Templeton et al., 1971; Chipman, 1972; Rice and Baptist, 1974). These reviews indicate that fish, particularly their eggs and larvae, are more sensitive to radiation than other marine species, although the actual dose rate required to produce observable effects on individuals from radionuclides dissolved in seawater are in dispute (Polikarpov, 1966; Templeton et al., 1971). Ophel et al. (1976) generalize from these published laboratory data that a minimum acute dose of about 100 rads is required to produce some mortality in a population of marine organisms. In addition, they estimate that a chronic dose of at least one rad day^{-1} (40 mrad hr^{-1}) is required to produce observable physiological effects in marine organisms. At field or experimental dose rates below this, they indicate that observable effects are masked by inherent biological variation. Shekhanova (this proceedings), however, states that effects on reproductive capacity of fish have been observed at doses ranging from 0.1-0.3 rad day^{-1}

Comparison of these estimates of minimum dose rates required to produce observable effects on individual organisms in laboratory experiments to maximum estimates of environmental dose rates listed in Table 1 indicates that present levels in the ocean are significantly below most levels shown to affect marine organisms adversely. Maximum dose rates from fallout range from 4.6×10^{-4} mrad hr^{-1} in crustacea to 0.147 mrad hr^{-1} in zooplankton. These rates are 270 to 87,000 times less than the estimated minimum dose rate of 40 mrad hr^{-1} discussed above. In the North Irish Sea, however, estimated maximum dose rates from radionuclides released from the nuclear fuel reprocessing plant at Windscale ranged from 3.9×10^{-3} mrad hr^{-1} for pelagic fish to 6.9 mrad hr^{-1} for zooplankton. This maximum estimate is within a factor of six of the one rad day^{-1} value and similar to the values obtained by Shekhanova.

Caution must be exercised, however, in extrapolating results from laboratory experiments to natural conditions. As indicated by Perez (this proceedings), duplication of natural conditions in the laboratory is extremely difficult and results obtained in artificial systems may be misleading.

TABLE 1. SUMMARY OF DOSE RATES (μrad h^{-1}) TO MARINE ORGANISMS FROM ENVIRONMENTAL RADIOACTIVITY (FROM WOODHEAD, 1973)

Source	Phytoplankton	Zooplankton	Mollusca	Crustacea	Fish	
	20 m depth, remote from sea bed	20 m depth, remote from sea bed	20 m depth, on the sea bed	20 m depth, on the sea bed	20 m depth, remote from sea bed	20 m depth, on the sea bed
NATURAL BACKGROUND						
Cosmic radiation	0.5	0.5	0.5	0.5	0.5	0.5
Internal activity	1.9-7.3	2.6-15.7	7.4-14.9	7.9-21.4	2.7-4.2	2.7-4.2
Water activity	0.4	0.2	0.1	0.1	0.1	0.1
Sediment activity, γ	-	-	1.5-16.0	1.5-16.0	-	1.5-16.0
β	-	-	1.6-21.0	1.6-21.0	-	1.6-21.0
Total	2.8-8.2	3.3-16.4	9.5-31.5	10.0-38.0	3.3-4.8	4.8-20.8
FALLOUT						
Internal activity, ^{3}H, ^{14}C, ^{90}Sr, ^{137}Cs, ^{239}Pu	0.01-0.88	0.23-13.4	0.06-0.32	0.004-0.097	0.02-0.06	0.02-0.06
Other nuclides	0.25-24.6	1.2-134	0.04-7.7	0.36	0.12-1.7	0.12-1.7
Water activity	5×10^{-5}-0.016	4×10^{-5}-0.011	$(0.2-32)\times10^{-4}$	$(0.2-32)\times10^{-4}$	$(0.3-65)\times10^{-4}$	$(0.2-32)\times10^{-4}$
Total	0.26-24.5	1.4-147	0.10-8.0	0.36-0.46	0.14-1.8	0.14-1.8
WASTE DISPOSAL						
Windscale						
Internal activity	200-2100	530-6900	15.3-58.9	6.9-67.9	0.5-1.5	0.5-1.5
Water activity	0.2-3.3	0.2-3.0	0.05-1.2	0.05-1.2	0.09-2.4	0.05-1.2
Sediment activity, γ	-	-	36.4-3340	36.4-3340	-	36.4-3340
β	-	-	207-5380	207-5380	-	207-5380
Total	200-2100	530-6900	51.8-3400	43.3-3410	0.6-3.9	37.0-3340
Bradwell						
Internal activity	-	-	1.37-1.81	-	-	-
Sediment activity, γ	-	-	1.69	1.69	-	1.69
β	-	-	1.32	1.32	-	1.39
Total	-	-	3.1-3.5	1.7	-	1.7

DETECTION OF ECOLOGICAL EFFECTS RESULTING FROM RADIOACTIVE CONTAMINATION

The detection of ecological changes caused in a marine ecosystem by the presence of a contaminant such as radioactivity is perhaps the most difficult type of assessment discussed in this paper. Our knowledge of ecological systems is not precise enough to predict what processes or structural characteristics would be most likely affected by radiation and which therefore should be monitored.

In the marine environment, three areas have received relatively large quantities of radioactivity as a result of man's activities. These are the Irish Sea, Columbia River estuary and adjacent coastal waters, and Central Pacific proving grounds. These areas have been the subject of relatively intense environmental studies (Lowman, 1960; Osterberg et al., 1974; Pruter and Alverson, 1972; Pentreath et al., 1973; Cross et al., 1975; Templeton et al., 1971). No gross ecological change that could be related to the presence of artificial radionuclides has been observed in the environment. Gorbman and James (1963), however, were able to detect radiation damage in thyroids of fish collected at Eniwetok Atoll at one-month and eight-month intervals after a nuclear explosion. No attempt was made to predict what effect this damage had on the population of fish living near the atoll.

Failure to detect radiation-induced changes at either population or ecosystem levels in these environments cannot be used as an argument that no effects have occurred. Instead it may reflect the superficiality of our understanding of how the system operates. We can only state that no catastrophic mortalities have occurred and more subtle changes which we might measure could be due to long-term fluctuations in the ecosystem.

We can infer, however, the possible effects of radiation at the population level using knowledge of the population dynamics of commercial fish species. This subject was examined by a recent International Atomic Energy Agency Panel studying "Effects of Ionizing Radiation on Aquatic Organisms and Ecosystems" (Templeton et al., 1976). The panel considered the role of density-dependent mortality in the stock-recruitment relationship in marine populations of both high and low fecundity (Fig. 1). Using commercially-exploited fish stocks as an example, the authors concluded that "if mortality of eggs is being enhanced by the low levels of irradiation presently existing in the marine environment, then recruitment to the stocks of highly fecund marine species of fish is unlikely to be adversely affected unless those stocks are already at risk because of severe over-exploitation." In other words, the result of mortalities of eggs caused by irradiation would decrease larval competition for food and space and, therefore, would increase the probability of survival for the remaining individuals.

Survival rates for highly fecund density-dependent stocks increase dramatically at low stock sizes (Fig. 2). The curve demonstrates the relationship between spawning stock size and survival for Atlantic menhaden for the 1955-1970 year class. Each dot on the graph shows the estimated egg production and percent survival to age one by year. In this case, the decrease in egg production of the spawning stock was caused by severe exploitation by the fishing industry and adverse environmental conditions. We would expect the same population response, however, if some perturbation such as radiation was causing mortalities of eggs and larvae. Obviously we would not expect the density-dependent relationship to compensate for radiation-induced mortalities in severely exploited fish stocks.

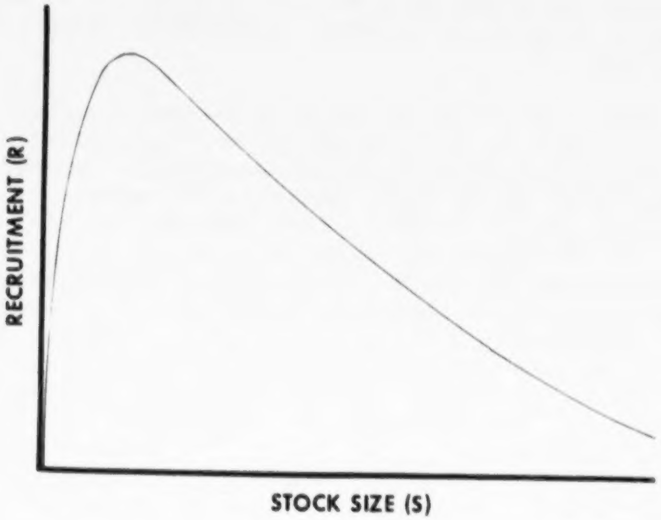

Figure 1. Density-dependent relationship between spawning stock size and recruitment in highly fecund species (figure from Beverton and Holt, 1957)

In addition, evidence is presented which indicates that increased pressure on fish stocks by over-exploitation or other stresses is compensated for by an increase in fecundity of surviving adults. In this manner, fish stocks have survived total mortality rates of 60-70% a year. Blaylock (1969), for example, reported that the mosquito fish, *Gambusia affinis*, increased fecundity relative to controls in the presence of chronic exposure to radioactivity in a freshwater environment.

The inherent dynamics of highly fecund marine populations, therefore, would compensate for additional mortalities of young caused by contaminants such as radionuclides. This compensating mechanism is limited in its capacity to "protect" a species which experiences high mortalities in its early stages. The actual number of mortalities that must occur to affect a population significantly would be highly variable and dependent on a number of additional factors such as predation, food supply, exploitation, etc.

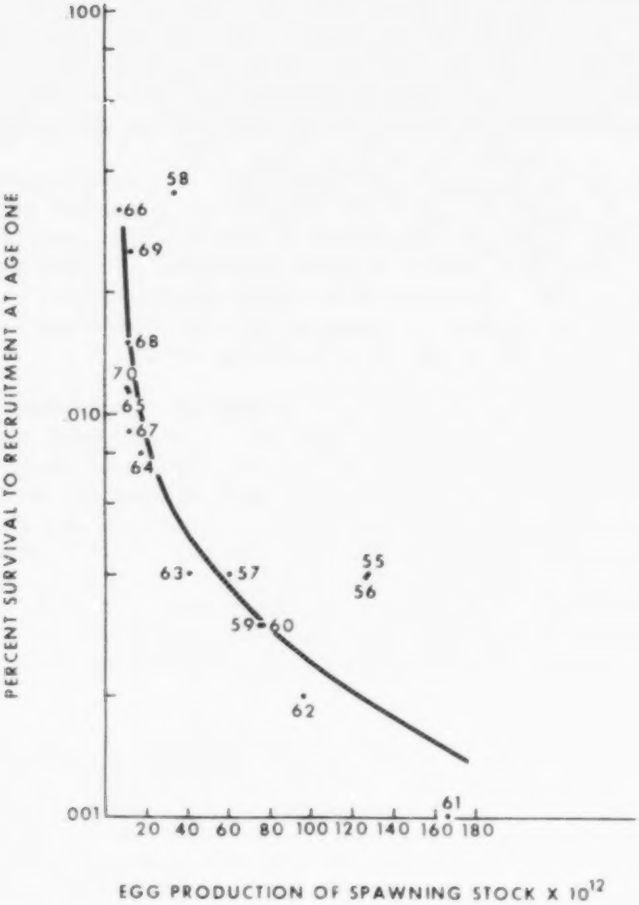

Figure 2. Relationship between spawning stock size and survival for Atlantic menhaden (*Brevoortia tyrannus*) for 1955-1970 year classes. (W. Nelson, personal communication)

CONCLUSIONS

The foregoing discussion indicates that man's introduction of artificial radionuclides into oceans has not caused significant adverse effects on marine populations. Within the scope of our knowledge, measurable changes in marine populations or ecosystems have not been observed in regions where artificial radionuclides have been released. In addition, most estimates of dose rates to aquatic organisms from releases of radionuclides are comparable to natural background dose rates and are significantly below dose rates most frequently shown to cause measurable damage to organisms in the laboratory. Although these discussions have centered on somatic effects, available evidence also indicates that no adverse genetic effects have occurred (Purdom, 1966; Templeton et al., 1976).

If nuclear weapons testing in the atmosphere is not resumed on a major scale, the oceans as a whole probably experienced their highest concentrations of artificial radionuclides in the early 1960's. Since the Nuclear Test Ban Treaty was signed, concentrations of fallout radioactivity have decreased significantly and no widespread contamination has resulted from nuclear power plants or nuclear-powered ships (Joseph et al., 1971). As long as existing levels of radioactivity in the oceans are kept at levels tolerable for man, harm to marine organisms is expected to be minimal or non-existent.

I have two major concerns for the future: catastrophic accidents and plutonium. The rupturing of a nuclear reactor in a coastal power plant or on a ship could release large quantities of radioactivity over a relatively large geographical area and expose marine organisms in this area to dose rates significantly higher than those known to cause mortalities. It is hoped that the present concern for safety in construction and operation of nuclear reactors will continue and such an event will never occur.

Because of its emission of alpha particles, long residence time in tissues and lack of a stable isotope, plutonium is the most toxic radionuclide known to man. Its usefulness to industrial and military establishments is causing the world-wide inventory of plutonium isotopes to increase significantly (National Academy of Sciences, 1975). Increased use of this material enhances the probability that additional uncontrolled releases to the marine environment will occur. A study panel for the U.S. National Academy of Sciences (1975) recommended: "Releases of transuranic elements to marine environment should be kept to a minimum. Any releases should be monitored, and plans for significant increases above current levels should be carefully scrutinized and regulated."

Although man has not released concentrations of radionuclides that have adversely affected marine populations, constant vigil must be maintained to assure that future uses of nuclear power do not cause widespread contamination. As our understanding of ecological processes improves and as experimental techniques are refined, research on the effect of irradiation from low chronic dose rates may disclose effects that are presently undetectable.

ACKNOWLEDGMENTS

I would like to acknowledge Dr. David Engel and Dr. Walter Nelson of this laboratory for their helpful suggestions in the preparation of this manuscript.

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THE EFFECT OF RADIOACTIVE POLLUTION OF RESERVOIRS ON FISH

by

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The distinguishing feature of contemporary pollution of the water with artificial radioactive materials consists in the predominant localization of these materials in the coastal and shelf zones of the ocean, in inland seas, in rivers and lakes, i.e., in regions of increased biomass and productivity. The possibility of increasing the concentration of artificial radionuclides in these regions is growing in connection with the existing practice of open dumping of low activity waste products in coastal regions of seas, bays and inland reservoirs. The results of multiple measurements of levels of the content of radioactive products in reservoirs of different kinds conducted in a number of studies confirm this thesis.

Today, the concentration of strontium-90 in coastal waters of the World Ocean comprises 0.1-0.2 pCe/l, in the inland and marginal seas 0.5-1.5 pCe/l, in rivers 1.5-5 pCe/l, and in lakes 3.5-12 pCe/l. In a number of reservoirs in which the radioactive wastes of nuclear industries are discharged, cumulative beta-activity fluctuates from 25 to 2000 pCe/l. A high concentration of certain radionuclides, including cesium-137, has been noted in bottom sediments.

Expansion of the scales of utilizing nuclear energy entails a proportional increase in the amount of radioactive wastes, part of which will unavoidably enter the reservoirs. The developing situation is stimulating an attentive analysis of the biological consequences that may result from the increased content of artificial radionuclides in reservoirs and determine the threshold of permissible irradiation of fishes at different stages of ontogenesis.

The biological effect during prolonged habitation of fish in the radioactively polluted environment is due to the power of absorbed dose and to the cumulative dose of radiation. At all stages of ontogenesis the power of the dose of radiation of the fish with the presence of artificial radionuclides in reservoirs consists of the external and internal sources. Sources of internal radiation are the radionuclides that are incorporated in given organs and tissues. Sources of external radiation are the radionuclides contained in the water, accumulated by aquatic plants, and absorbed in the bottom deposits. Additionally, radiation resulting from the radionuclides in neighboring organs or tissues that are characterized by a high degree of accumulation can be external with respect to some particular organ.

Dose power of internal radiation is determined by the intensity of accumulation of radionuclides in the organs of the fish and by their energy of radiation. The dose power of external radiation is closely dependent on the ecology of fish.

Calculations have shown that the power of the dose of radiation of ocean and sea pelagic fishes resulting from artificial radionuclides today is 2-3 orders smaller than the dose power of radiation from natural sources of ionizing radiation. In rivers the doses of radiation of fish from natural and artificial sources are comparable. In lakes, especially if cesium-137 is present in them, the dose of radiation of bottom fishes from artificial sources is 5-10 times higher than that from natural sources. In reservoirs in which low activity wastes are discharged, dose power of radiation of fish resulting from artificial radionuclides fluctuates within limits of 0.1-1 rad/day and sometimes reaches even higher levels.

Natural radiation loads pertain to the constantly acting factors of the environment, to which all living organisms have developed corresponding radioresistance over the course of prolonged evolution. Consequently, there is no basis to assume the possibility of the harmless effect of artificial radionuclides on ocean and marine pelagic fishes with the doses of radiation that presently exist because of them. Certain changes in the biological condition of fish can exist in fresh water fish and fish that inhabit stretches of seas regularly polluted by discharges of low activity wastes.

In coastal areas of the seas a large number of valuable commercial species of fish spawn: the herring, the Baltic herring, the whitefish, the sole and many others. The fry of these and other species of fish also inhabit these areas for a long period of time. By living in the aquatoria polluted by radioactive wastes, they are exposed to radiation at the most radiosensitive period of ontogenesis--in the period of the establishment and development of all organs and systems.

The problem of the biological effect of small doses of ionizing radiation on living organisms is extremely complex. The scale of danger created by chronic irradiation with low dose power cannot be predicted based on experiments with acute irradiation. Conclusions regarding the harmful consequences of radioactive pollution of the environment should come from experiments with chronic continuous irradiation simulating the conditions that are created for fish in reservoirs. This tenet was taken as the basis for determining the effect of radioactive pollution of the water on fish. Functional changes in different organs and systems and their structural changes were used as indicators.

Of the large number of investigations that have been devoted to identifying the effect of ionizing radiation on the fish, from the viewpoint of predicting the biological consequences of pollution of reservoirs with radioactive products, those are of interest that characterize deviations from the normal in a range of doses that actually exist or are possible with consideration of the scale of development of nuclear energy. In regard to this we shall analyze materials obtained during the chronic irradiation of fish with a dose power of 0.1-1 rad/day.

The capacity for reproduction, fertility and the quality of the spawned generation are the most significant indicators of the well-being of any living organism. They determine in fact the numbers of the population and the replenishment of the school of fish. It has been established that at all stages of ontogenesis prolonged irradiation of the sexual organs with a dose power of 0.1-0.3 rad/day causes disruption of the process of formation and function of reproduction. The morphological structure of the primary germ cells and their time of appearance are similar in the irradiated and intact specimens. The prolongation of the miotic cycle of the primary germ cells that leads to a delay in sexual differentiation is noticeable. At the later stages of gametogenesis, the changes caused by irradiation appear more clearly.

Chronic irradiation of the testicles leads to biochemical changes that are expressed in a reduced content of glycogen and an increase in the content of fat. In the generative tissues cells appear with pycnoticlysis of the nucleus.

The ovaries of fish, like those of the warm-blooded animals, are more radioresistant than the testicles. In the process of differentiation and development of the oocytes, irradiation of the ovaries with a dose power of 0.1-0.3 rad/day entails a change in the volume of the nucleus and protoplasm and the nuclear-plasma ratio. There is a reduction in the number of young oocytes, and as a result relative fertility of the irradiated females becomes 1.5 times less than normal. The generation obtained from fish irradiated in the indicated range of doses is characterized by reduced viability.

An increase in the dose power of radiation of the gonads to 1-3 rad/day leads to more profound changes. Under these conditions the males lose their capacity for reproduction after irradiation of the testicles in a dose of 550-660 rad, and the females following irradiation of the ovaries with a dose of 1000-1200 rad.

The functional condition of the endocrine system, specifically, gonadotropic activity of the hypophysis, is intimately related to the reproductive capacity of the fish. A histological investigation has shown that during chronic irradiation of the hypophysis with a dose power of 0.1-0.3 rad/day, growth of the hypophysary parenchyma is observed and the process of secretion of the hormone into the blood stream is disrupted. With an increase in dose power the effect of the action increases.

A reduction in the resistance of irradiated fish to parasitic and infectious diseases has been established. This reaction is due to changes in the hematopoietic process, the morphological composition of the blood and the immunological reactiveness. In embryos of fish during chronic irradiation of the axial cellular mass, responsible for hematopoiesis, it is more weakly pronounced than in the intact ones. The process of differentiation of the mesenchyme cells into hemocytoblasts is inhibited, and the intensity of hematopoiesis decreases on the periblast as well. Under the effect of irradiation there is an increase in the number of erythrocytes with morphological disruptions and there is an increase in the number of pathological mitoses. Retardation is noted in the formation of the pronephric glomeruli. The number of leucocytes decreases, which leads to inhibition of phagocytic activity. Changes in the process of hematopoiesis have a phase character and depend on the power and total magnitude of the absorbed dose. A decrease in the immunological reactiveness in fry is noted upon irradiating the kidneys with a dose of 4-5 rad with a dose power of 0.05-0.1 rad/day.

Hence, a clearly unfavorable situation for fish is developing in the aquatoria polluted with radioactive wastes. The doses of radiation that form there entail a reduction in the viability of fish and disruption of reproductive function. It does not seem possible to block off contaminated stretches of the sea from the entry of fish into them from the purer regions. Proportional to expansion of the scale of utilizing nuclear energy and the increase in the amount of radioactive wastes discharged into reservoirs, the area of polluted stretches will grow proportionally. This should be considered when developing forecast monitoring.

A NON-STANDARD APPROACH TO HETEROTROPHY:
ATP ESTIMATION OF NATURAL POPULATIONS OF SELECTIVELY-FILTERED BACTERIOPLANKTON AND THEIR GROWTH RATES ON IN-SITU WATER IN DIFFUSION-CULTURE[1]

by

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ABSTRACT

The sieve-like properties of the Nuclepore membrane filter are utilized to separate the bacterioplankton from the larger plankton, and its biomass is determined by ATP assay. Diurnal growth of the bacterioplankton is observed in a chamber with a Nuclepore membrane wall designed to cage the microorganisms while allowing naturally occurring nutrients to diffuse freely into the culture. The results obtained at a blue-water station east of the Azores in August 1975 are presented to show the kind of information that can be obtained. The bacterioplankton biomass varied from 1.6 to 8.4 mg C/m^3 over the uppermost 250 m, and its distribution was strongly correlated to that of phytoplankton and dissolved carbohydrates. Bacterioplankton exposed in growth chambers to water continuously pumped from 50 m (oxygen maximum) and 80 m (chlorophyll maximum) exhibited diurnal growth patterns from which daily production was estimated to be 20 to 67 mg C/m^3. A calculated mean annual production of 13 g C/m^3 is some three times the mean obtained by other methods.

INTRODUCTION

A first approximation of the functional role of a class of organisms within an ecosystem requires a knowledge of both its biomass and rate of production. The development of procedures for such studies of bacterioplankton would permit observations on the distribution of biomass, factors that control its rate of production, and evaluation of its role in the in-situ transformation of organic carbon. Obtaining such data for any group of organisms is

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[1] This study was supported by Grant DES 74-01537 from the Biological Oceanography Program of the National Science Foundation.

difficult, but especially so for the bacterioplankton due to their small size, small biomass, and rapid adaptation to changing conditions. However, these very attributes can be used to advantage in designing new methods such as those developed in this laboratory over the past several years. They include a procedure for the biochemical estimation of the biomass of bacterioplankton as well as a direct method for estimating growth rates and growth patterns in situ. Although these techniques are still under development, we feel that they show promise as a method for studying bacterioplankton in both natural and pollution-stressed planktonic ecosystems. They offer alternatives to the direct microscopic counting procedures for biomass and the ^{14}C indirect method of growth rate measurement used by Sorokin (1971) for characterizing bacterioplankton in the Pacific Ocean. During R/V *Trident* Cruise 170 in the North Atlantic from Rhode Island along the shelf to the Grand Banks and across the Atlantic via the Azores to Spain, a spectrum of water masses was examined by these procedures. To illustrate the type of information that can be obtained, we report here the results of one station. Station 13 was a 4,450 m deep bluewater station east of the Azores at 36°59'N, 21°22'W that was occupied for 36 hours commencing at 0800 local time 14 August 1975.

We gratefully acknowledge the assistance of all members of the scientific party of TR-170, especially Kenneth R. Hinga and Paula J. Willis for ATP determination, Fred W. French III for pigment determinations, Kenneth M. Johnson and Curtis M. Burney for carbohydrate determinations, and James Hannon for marine technical services.

METHODS

BIOMASS ESTIMATION BY ATP

Samples of seawater are drawn through Nuclepore filter membranes (Nuclepore Corp., Pleasanton, Calif.) by a 100 mg Hg vacuum. The ATP of microorganisms retained on the filter is extracted and assayed with a procedure similar to that of Holm-Hansen and Booth (1966). The filter is immersed in 5 ml of boiling Tris (hydroxymethylaminomethane) buffer (pH 9.0 at 20°C) and extracted for two minutes. The extract is then immediately frozen until assayed aboard ship at the close of the station, using a DuPont Luminescence Biometer (E. I. DuPont de Nemours Co., Wilmington, Del.) to measure the luciferin-luciferase light reaction (Allen, 1972).

Differentiation between the ATP of bacterioplankton and that of larger particles is accomplished by selectively filtering the 1,000 μm prescreened sample first through a 3 μm Nuclepore filter and then through a 0.2 μm pore size Nuclepore. The filters are then extracted for ATP. Since the Nuclepore filter functions as a microscopic sieve (Sheldon, 1972), the two fractions represent the ATP of particles between 3 and 1,000 μm and that between 0.2 and 3 μm. Replicate water samples of 145 ml were taken from 5 and 30 liter Niskin bottles of PVC plastic which were washed with 0.1 N HCl just prior to being lowered to depth (Sorokin, 1971).

ESTIMATION OF GROWTH RATES

By applying the bioengineering principles for the dialysis culture of microorganisms described by Schultz and Gerhardt (1969), we developed a culture chamber (Fig. 1) in which a study population of microorganisms can be held captive and yet be exposed to dissolved natural substrates at natural concentrations (Lavoie, 1975). A 0.1 μm pore size Nuclepore membrane is used as a barrier to retain the bacteria while soluble substrates are allowed to diffuse into the culture from a continuously replenished volume of seawater and metabolic by-products are able to diffuse out. Theoretical considerations indicate that substrate concentration and rate of biomass increase within this culture device should closely simulate the natural situation (Lavoie, 1975). This is achieved by the chamber configuration and a vigorous agitation of the liquid adjacent to both the membrane surfaces which promotes a rapid transfer of solutes across the membrane. The population therefore responds rapidly to changes in the quality and quantity of substrate in the seawater, thus enabling the observation of natural growth patterns.

To inoculate the chambers, seawater from the 50 and 80 m depths (the oxygen and chlorophyll maxima respectively) were aseptically filtered through a 3 μm pore size Nuclepore filter, and 180 ml portions were put into each previously autoclaved culture device. Seawater was drawn up continuously from 50 m and 80 m depths through two polyethylene tubes, 0.6 mm inside diameter. A high speed peristaltic pump provided water at approximately 500 ml/min to a continuously overflowing 2-liter reservoir bottle for each depth while tubing from each bottle supplied water at a rate of about 100 ml/min to the medium chambers of each of three replicate culture devices. The chambers were immersed in a flowing water bath in the dimly lit wet lab, but temperature and light conditions were less than ideal. At four-hour intervals duplicate samples were drawn from each chamber and assayed for ATP concentration.

OTHER PARAMETERS

Several other measurements were made for supportive data. Of those reported here, chlorophyll *a* and phaeopigments were measured by fluorometry (Yentsch and Menzel, 1963; Holm-Hansen et al., 1965); dissolved oxygen was measured by the standard Winkler titration; carbohydrates were measured as hexose equivalents by a technique recently developed in our laboratory (Johnson and Sieburth, 1976; Burney and Sieburth, 1976). Water from the upper 150 μm of the air-sea interface was obtained with a nylon screen sampler (Sieburth et al., 1976).

RESULTS

In Figure 2 the vertical distribution of the two fractions of particulate ATP is plotted on the left. Pigments, dissolved carbohydrates, and dissolved oxygen are superimposed on the same depth scale to show their relative distributions. For the smaller than 3 μm fraction, three ATP peaks are immediately obvious: at the sea-air interface, at 50 m (oxygen maximum) and at 80 m (chlorophyll maximum). All points except those at 30 and 40 m are

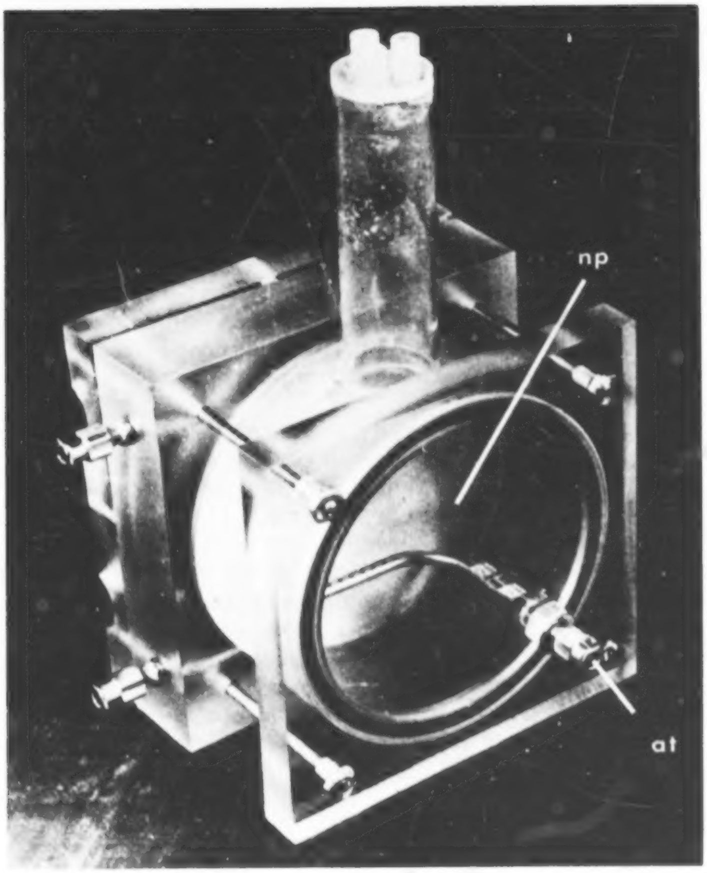

Figure 1. Diffusion culture apparatus: view into the 180 ml growth chamber. Filtered air is pumped into the culture for aeration and mixing via the aerator tube (at) and to provide turbulence at the membrane surface to promote diffusion. A Nuclepore membrane (np) of 0.1 μm porosity allows diffusion of seawater solutes into the culture while keeping the bacterioplankton population captive. Hidden by the membrane in the photograph is the 100 ml medium chamber, through which untreated seawater is continuously pumped by means of the two ports seen on the left side. The medium chamber is agitated by a teflon-coated magnetic bar to provide turbulence at the membrane surface. Approximate dimensions of the assembled chamber are 10 cm on each axis.

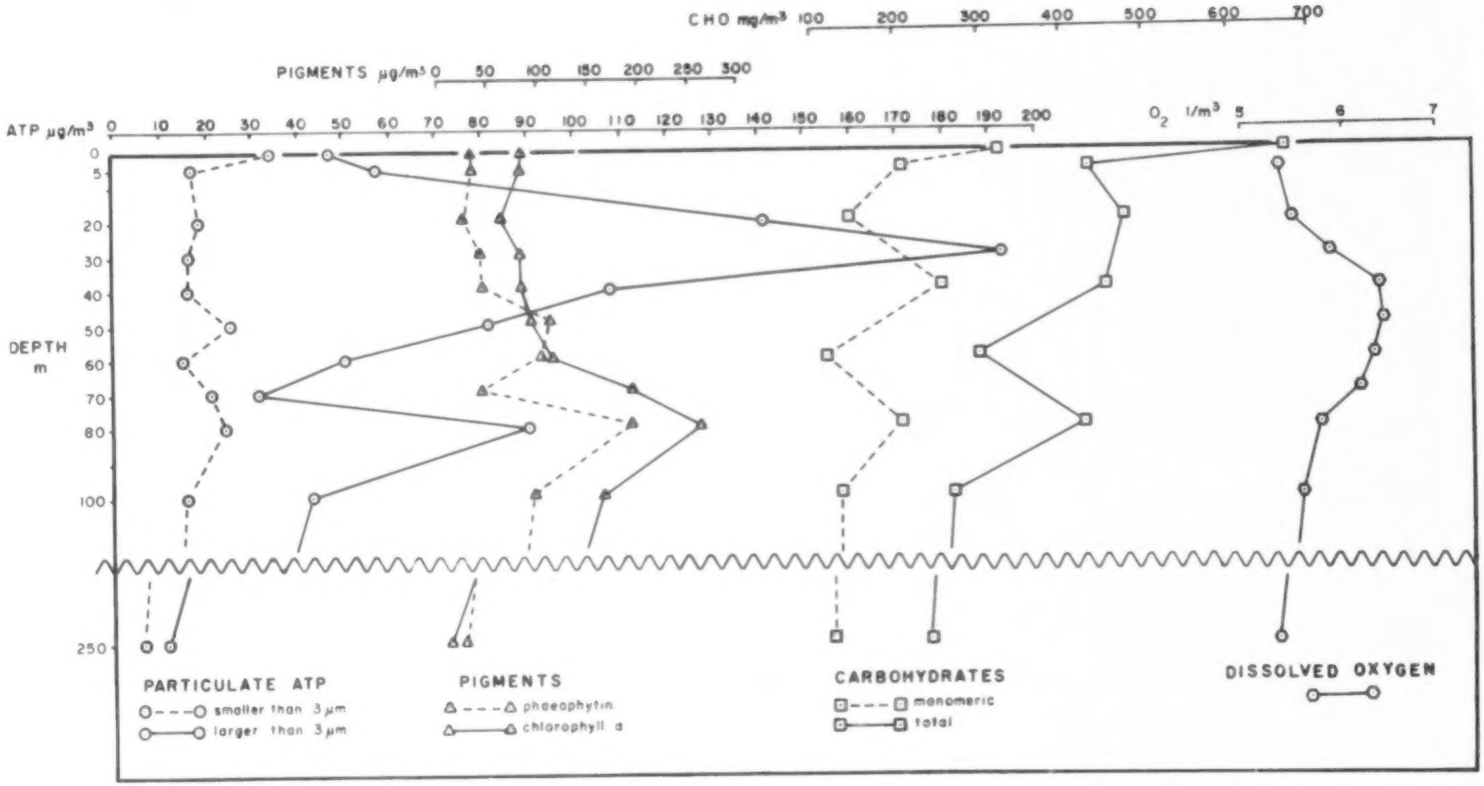

Figure 2. Depth distribution of particulate ATP and other parameters at Station 13, R/V *Trident* Cruise 170 at 1000 hrs local time, 14 August 1975, at 36°59'N, 21°22'W. The bacterioplankton is the smaller than 3 µm ATP fraction.

statistically different from each other at the 1% confidence level. The significance of this distribution of bacteria-sized particles becomes clearer when the other parameters are examined. The larger particulate ATP fraction shows a more extreme distribution as might be expected. The organisms constituting this fraction evidently were stratified principally at 30 m and 80 m. The large peak at 30 m, with a minimum chlorophyll *a* concentration, implies a preponderance of micro-zooplankton dominated by protozoa, while the maximum for chlorophyll *a* at 80 m indicates the major zone of phytoplankton. At all sampling depths the ATP-biomass of this fraction was greater than the less than 3 μm fraction. It is perhaps more interesting to view this fact from the opposite perspective, that is, that the less than 3 μm fraction at times makes up more than 30% of the total biomass of microplankton less than 1000 μm in size. Figure 3 plots the mean growth obtained in diffusion cultures of the smaller than 3 μm fraction exposed to water drawn from 50 m (oxygen maximum) and 80 m (chlorophyll maximum). The diurnal growth pattern is consistent with the thesis that these microorganisms utilize dissolved substrates that originate with the primary producers. These substrates may be released either directly, through exudation during photosynthesis, or indirectly, through grazing by zooplankton, with attendant cell damage and zooplankton excretion. The depth distribution of the bacterioplankton is given in Table 1 while the amount of bacterioplankton produced in diffusion culture at the maxima at 50 and 80 m is presented in Table 2. The apparent daily production for these microzones of intense activity ranged from 21 to 67 mg C/m^3 which on an annual basis would yield 7.6 to 24.5 g C/m^3 with a mean of 13 g C/m^3. This compares with a range of 1 to 6 g C/m^3 estimated for the Pacific and the North Sea by Sorokin (1971) and J. Meyer-Reil (personal communication), respectively.

TABLE 1. DEPTH DISTRIBUTION OF SMALLER THAN 3 μm PARTICULATE ATP AND COMPUTED CELLULAR CARBON AT STATION 13, R/V *TRIDENT* CRUISE 170, 1000 HRS, 14 AUGUST 1975, 36°59'N, 21°22'W. MEAN VALUES ±95% CONFIDENCE LIMITS.

Depth	[ATP] μg/m^3	(cellular carbon) mg/m^3*
0	33.7 ± 0.49	8.4 ± 0.12
5	16.8 ± 0.09	4.2 ± 0.03
20	18.4 ± 0.23	4.6 ± 0.06
30	16.7 ± 0.25	4.2 ± 0.06
40	16.7 ± 0.18	4.2 ± 0.05
50	25.8 ± 0.55	6.5 ± 0.14
60	15.3 ± 0.23	3.8 ± 0.06
70	21.6 ± 0.10	5.4 ± 0.03
80	24.4 ± 0.16	6.1 ± 0.04
100	15.6 ± 0.21	3.9 ± 0.05
250	6.4 ± 0.16	1.6 ± 0.04

*Computed as follows: [ATP] μg/m^3 x 0.25 = (cellular carbon) mg/m^3 (from Hamilton and Holm-Hansen, 1967).

TABLE 2. ESTIMATED INCREASE IN BACTERIOPLANKTON OCCURRING AT THE OXYGEN AND CHLOROPHYLL MAXIMA (50 and 80 meters) AT STATION 13, TR-170, AS DETERMINED BY DIFFUSION CULTURE

Depth and Growth Periods	Generations x	Initial [ATP] $\mu g/m^3$ =	Increase in [ATP] $\mu g/m^3$		Computed Increase in cell carbon mg/m^3*
50 m, oxygen maximum					
First growth period					
18-22 hrs	1.23	92	113		28
				period total	28
Second growth period					
02-06 hrs	0.37	120	45		11.1
06-10 hrs	0.26	150	39		9.7
				period total	20.8
80 m, chlorophyll maximum					
First growth period					
14-18 hrs	0.63	89	55		13.7
18-22 hrs	0.43	138	59		14.7
				period total	28.4
Second growth period					
02-06 hrs	0.42	165	69		17.2
06-10 hrs	0.92	220	200		50.0
				period total	67.2

*[ATP] $\mu g/m^3 \times 0.25$ = [cellular carbon] mg/m^3 (from Hamilton and Holm-Hansen, 1967).

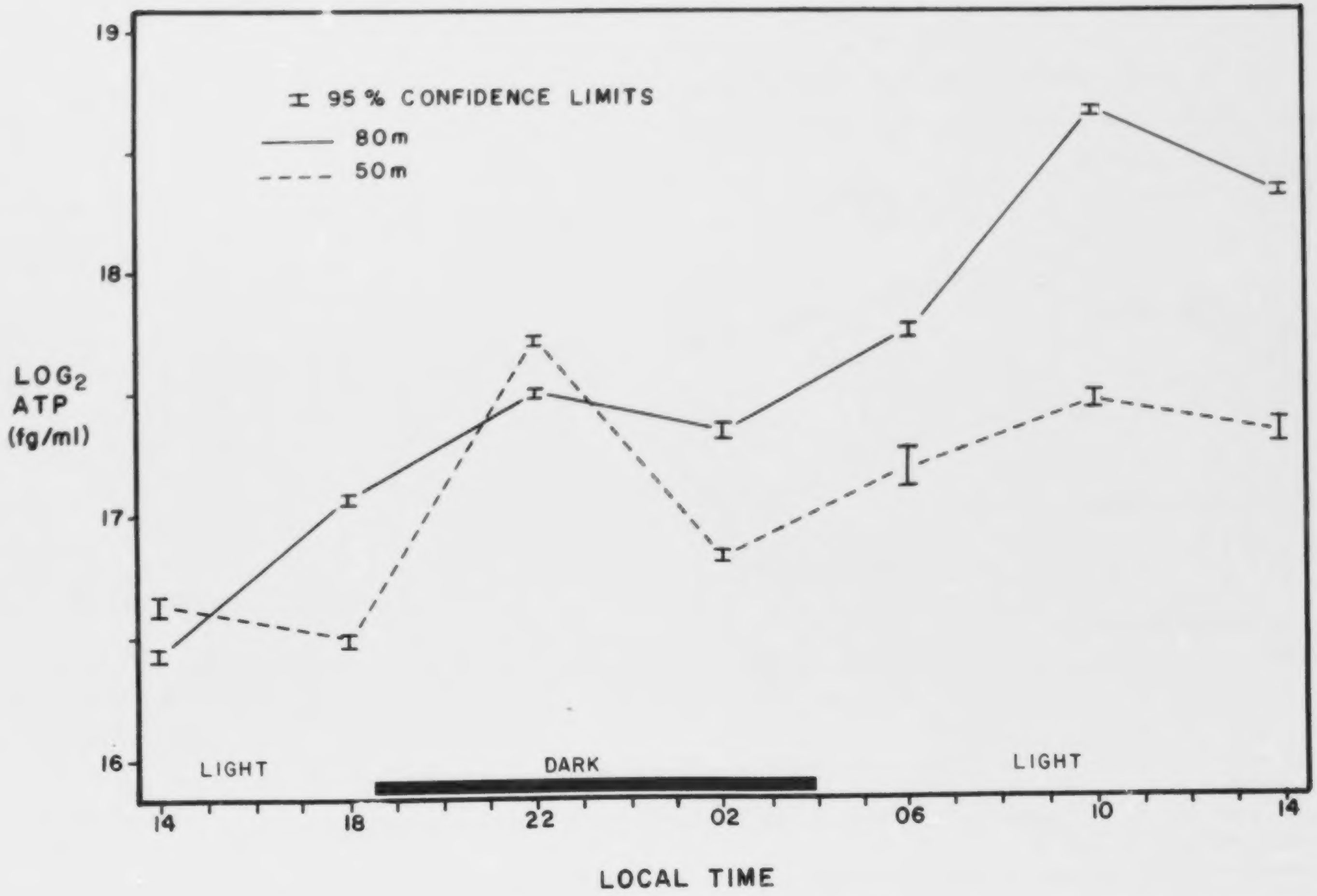

Figure 3. Growth patterns of bacterioplankton populations held 24 hrs in caged culture apparatus while exposed to untreated seawater continuously pumped from 50 and 80 m. Each point represents the mean of particulate ATP in 3 replicate cultures. Ordinate scale is expressed as $\log_2$ fg ATP/ml (1 fg = 10^{-15}g), and an increase in one unit indicates a doubling of ATP concentration.

DISCUSSION

The results for estimating biomass and productivity presented here are preliminary in nature. Some of the assumptions and the procedures described require further testing before the validity, limitations, and thus the usefulness of this methodology can be determined. The 3 μm particle size cutoff was chosen arbitrarily but appears to be a fortunate choice (Hoppe, 1976).

Laboratory studies using the diffusion culture device (Lavoie, 1975) show that when a fraction smaller than 3 μm is removed from the natural light regime, its growth is closely coupled to the quality and quantity of nutrients in the diffusion medium. By eliminating predation and other removal mechanisms, any growth in the population is cumulative and is directly proportional to the amount of substrate in the diffusion medium. The production observed in the open ocean experiment implies that a substantial amount of the primary production is converted to bacterial biomass.

The distribution and rates of growth of the bacterioplankton seem to agree with the data on dissolved organic matter and the occurrence of protist biomass. The 50 ATP peak occurs at the oxygen maximum, where presumably most of the primary production is occurring (9% light level). Phaeopigments also peak at this depth, indicating some phytoplankton cell decomposition, due either to autolysis or to grazing by zooplankton (Lorenzen, 1967). Either process would provide soluble substrates for bacterial growth, as would exudation by intact cells (e.g., Thomas, 1971; Smith and Wiebe, 1976). The actual presence of dissolved substrates is substantiated by the observed carbohydrate concentration which has a maximum at this depth.

The 80 m peak in the smaller than 3 μm particulate ATP distribution is also accompanied by a higher peak in the phaeopigments and another maxima for dissolved carbohydrates. This depth is characterized by chlorophyll *a* maximum (at the 1% light level) indicating the maximum for phytoplankton biomass.

Before annual productivity values per m^2 can be estimated for specific oceanic areas, a finer profiling must determine the rates of productivity outside the patches of plankton accumulation observed in this study where maximum rates presumably occur.

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THE DEVELOPMENT OF STANDARD METHODS OF MEASURING MICROBIOLOGICAL PRODUCTION

by

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The task of this report is to present suggestions concerning a unified plan of research involving the determination of microbe production in aquatic ecosystems.

It is unnecessary to repeat that the role of microorganisms as great utilizers and converters in the life of any natural community is vast and irreplaceable, in connection with which it is urgently necessary, first, to have a knowledge of, and secondly, to utilize the activity of microorganisms in natural biotopes. How can one estimate the character and scale of activity of microorganisms in the habitations that interest us? This is one of the basic problems of ecological microbiology of the present day. The approach to its solution was developed by Vinogradskiy, and is the basis of research in this region today.

Vinogradskiy developed the idea that, although certainly in any situation it is necessary to study the behavior of individual types of microorganisms, one cannot draw conclusions regarding their role in nature by basing one's self solely on data obtained during work with pure cultures. The investigation of the actual processes realized by microorganisms in nature should be included in the study of behavior of microbe societies in combination with all other (physicochemical, biological) components of the ecosystems.

The basic principles of microbiological ecology were formulated by Vinogradskiy:

1. One should determine the physical and chemical conditions in which the studied phenomenon occurs, related to the activity of microbes and determine which groups of organisms participate in it.
2. One should identify the representatives of groups of microorganisms that are important for the given phenomenon, and study their physiology and interrelationships with other organisms, i.e., determine their biological niche.
3. One should attempt to explain the true significance of their activity in nature (i.e., in a society with other species), approaching this from the quantitative aspect.

All of this signifies the need for combining laboratory investigations of pure and mixed cultures under conditions that simulate various natural situations with field research that includes chemical, physical and biological characterizations of the biotope, the characterization of the state of microorganisms and their interaction with each other and with organisms of the other levels of the ecosystem. Works of recent years have been developing in this very direction and this approach is recognized as a wise one by modern investigators.

The most important aspect that integrates the demands made upon research in the realm of ecological microbiology is the determination of the so-called microbe production.

THE CONCEPT OF "MICROBE PRODUCTION"

In a general sense, the concept of "microbe production" means the dynamics of microbial manifestations (the biomasses, population and functions) in the ecosystem. Functioning of the ecosystem is the cycle of substance and transport of energy whose significant aspect is a certain equilibrium between the synthesis of organic material and its destruction. The synthesis of material occurs at all levels of the ecosystem; if its dynamics can on the whole be termed production of the ecosystem, then dynamics of synthesis of organic material (as the result of which growth, reproduction and the liberation of organic materials into the environment occur), each component part of the ecosystem can be called production of the given member or level of the ecosystem. In each functioning community of organisms there is activity of microorganisms, and consequently, microbial production, i.e., the synthesis of material by microorganisms expressed in their accumulation of biomass or maintenance of biomass at a single level for a certain time under conditions in which attrition and consumption occur.

The activity of microorganisms occurs at all levels of the ecosystem; one should proceed from this when determining microbial production. If the subject concerns aquatic ecosystems, i.e,, the so-called free-living microflora: epiphyte microflora of the algac type, the microbe-decomposers, the heterotrophic bacteria that feed on organic wastes of the algae and that serve as food for the zooplankton, the chemosynthesizing and photosynthesizing bacteria, as well as the pathogenic and symbiotic microflora of all the higher and lower aquatic organisms. The pathogenic microorganisms can obviously play an important role in populations of fishes, etc. It is obvious that methods of determining the free-living and pathogenic microflora should be different. The destruction of dead organisms is an important element of action of the microorganisms and should also be investigated for all levels and components of the ecosystem.

It is initially important to bear in mind the following variations that are possible during the investigation of microbial production:

1. The system is supplied with biogenic elements and has suitable physico-chemical indicators for the development of microorganisms but does not have in general an adequate number of cells of the corresponding microorganisms that are capable of utilizing the given biogens. This is possible for ocean

water as the result of dilution, stirring or the absence of stirring. In this situation there is no production, which makes it necessary attentively to deal with the problem of the size of the inoculate of microorganisms. It is solved by determining the number of microorganisms of different species in a given place of habitation in combination with laboratory investigations of the initial values of the inoculate required for the beginning of growth under similar conditions.

2. Another possible situation is when one cannot measure the actual microbe production--microorganisms are present in the system, but there is an insufficient concentration of biogenic elements or unsuitable physico-chemical conditions for the beginning of their multiplication. In this case one can determine the potential activity of the group of microbes present in the water by adding different substrates to a removed sample and following their consumption and the increment in biomass (population), or the functioning of the microbes. In such systems, the microorganisms are in a state of the quiescent forms or in a state of low activity and readiness for reproduction.

3. With the presence in the system of a sufficient amount of biogenic elements and substrates for growth, the microorganisms reproduce, i.e., one can determine actual production. Its scales depend on the concentration of nutrients and consequently, low production can exist which is equal to the rate of mortality and consumption, as the result of which the total number of cells remains constant. This is the usual situation for unpolluted water. Upon enrichment of the medium, a flare of development, the so-called "flowering" of microorganisms is possible when a significant biomass of them accumulates in the system.

Apparently, there is a direct relationship between microproduction and the dynamics of functioning of the ecosystem on the whole. In a certain sense, microbe production can serve as an indicator of functioning of the ecosystems. Bearing all of the above in mind, one can state that during the determination of microbe production, in order to judge correctly the microbiological processes that are occurring in the aqueous ecosystem, one must know the following:

1. The chemical composition of the water (the content of P, N, and organic substances), the physico-chemical conditions, and what primary production was (in order to know the level of productivity of the water).
2. The composition and population of the living population of the given biotope. It is necessary to include all these in investigating the activity of microorganisms.
3. The general population and the population of specific groups of microorganisms.
4. Actual and potential microbe production in accordance with what sources of material and energy exist in the medium.

By knowing all of these data, one can on the one hand determine the scale of microbe production in the ecosystem, and, on the other hand, its actual value for the functioning of the ecosystem.

METHODS OF DETERMINING MICROBE PRODUCTION

Methods of determining microbe production, particularly in fresh water, are being intensively developed in the USSR. We shall list the most suitable methods of determining microbe production with brief commentaries.

1. Determining the increase in the number of bacteria in isolated water samples (Gak, 1975).

Samples of water taken with a sterile bathometer are poured into sterile glass dark flasks with a volume of 250 ml, with ground-glass stoppers. Half of the samples are filtered through a preliminary filter to separate the zoo- and phytoplankton. The total number of bacteria and the number of bacteria of the groups of interest are determined in the flasks containing filtered and unfiltered water. The flasks are lowered into a reservoir to the level from which the sample was taken or are incubated in aquaria in which conditions that approximate the natural ones have been created.

Exposure time is selected experimentally for reservoirs of different latitudes and different trophic types. It should be equal to the average time of bacterial generation. In the mesotrophic and eutrophic reservoirs of the middle latitutudes, G. W. Gak recommends daily exposure of the samples. Upon terminating exposure, the number of bacteria is once again determined. The constant of the rate of production in the filtered sample of water is determined according to the following formula (Gak, 1975).

$$K = \frac{\ln b_t - \ln b_o}{t} = \frac{2.303(\log b_t - \log b_o)}{t} \qquad (1)$$

where b_o and b_t are the initial and terminal concentration of bacteria in the filtered water.

The change in the biomass of bacteria is described by the equation

$$\frac{dB}{dt} = KB - K_1B \qquad (2)$$

where K_1 is the constant of the rate of consumption of bacteria and B is the concentration of bacteria in the unfiltered water.

$$K_1 = \frac{\log \frac{b_t}{b_o} - \log \frac{B_t}{B_o}}{0.4343\ t} \qquad (3)$$

$$Yt = K_1\overline{B} \qquad (4)$$

where $\overline{B}$ is the average biomass of bacteria over a time t, Yt is the consumption of bacteria. Then, from (3) and (4), the production of bacteria P_t is determined as

$$P_t = B_t - B_o + \overline{B}\,\frac{\log \frac{b_t}{b_o} - \log \frac{B_t}{B_o}}{0.4343\ t} \qquad (5)$$

or a simplified method of calculation

$$P_t = \overline{B}\,Kt \quad \text{[the same as (5)]}$$

2. The determination of microbe production by the radiocarbon method according to the rate of assimilation of carbon dioxide (Romanenko and Kuznetsov, 1974).

The rate of absorption of carbon dioxide by microorganisms in darkness in a reservoir is a cumulative value that forms as the result of chemosynthesis and heterotrophic assimilation of carbon dioxide. According to the calculations, about 6% of the biomass of heterotrophic bacteria forms at the expense of CO_2. Perceptible production of organic material as the result of chemosynthesis occurs only in limited cases, when one observes copious liberation of hydrogen sulfide as the result of anaerobic lysis in the eutrophic or meromictic reservoirs. In reservoirs with a normal oxygen regime in the layer of water, bacterial chemosythesis reaches significant values (0.05-0.2 g/m^3 of biomass per day), only in the bottommost layer of water and in the upper layer of the bottom sediments. In the meromictic reservoirs, where an anaerobic zone is constantly forming, the role of production of chemosynthesis grows. If the anaerobic zone is within limits of the illuminated zone, the intensive development of the photosynthesizing bacteria is observed in it.

Methods of separate determination of heterotrophic and chemosynthetic fixation of carbon dioxide have been described by Sorokin (1964, 1970). Only heterotrophic fixation occurs in the surface layer, and upon determining the vertical distribution of the heterotrophic bacteria it is considered that heterotrophic assimilation of carbon dioxide is proportional to this distribution. The value of assimilation of carbon dioxide in darkness in the layers adjacent to the anaerobic zone is determined. It usually exceeds assimilation in the surface layers by a value that corresponds to the chemosynthetic fixation of CO_2. One can define the values of chemosynthetic and heterotrophic fixation of CO_2 according to the increment in the biomass of bacteria by determining their population or production, taking into account that the biomass of the chemosynthesizing bacteria is wholly formed from CO_2, while only 6% of the biomass forms from CO_2 in the heterotrophic bacteria. Another method of measurement is made according to oxygen consumption, since the O_2/CO_2 ratio differs 10-20 times for these two processes.

3. In order to identify localization of active microbial populations and the effectiveness of bacterial biosynthesis in reservoirs, a method of determining so-called potential microbe production is employed.

In this case, a trace amount (1-100 μg/l) C-labeled dissolved organic substance is added to the samples of water from the reservoir and the relative activity of the bacteria for samples from different biotopes is judged according to inclusion of the label in the biomass, that is, the intensity of bacterial biosynthesis is indirectly characterized. The method has made it possible to identify the principles of localization of active microflora in a layer of water and in bottom sediments (Sorokin, 1970). The method was suggested by Parsons and Strickland (1962). One can also name certain other methods of determining microproduction, for example, the method of microcolonies, a method using overgrown glass, a method of determining production according to the rate of O_2 consumption in the reservoir or in an isolated sample of water (only suitable for waters rich in microflora).

A variety of methods using labeled substrates makes it possible to judge on the microbiological destruction of organic material, the activity of microflora that utilize methane, hydrogen, the sulfides, ammonia, etc.; the method of radioautography is used to count colonies of specific bacteria and to calculate the activity of individual cells. Attempts to use the principles of continuous cultivation of microbes for determining microbial production are interesting.

Brok and Brok (1968) studied the rate of growth of bottom algae, using a small stream as the container of a continuous culture. They covered a stretch of the river with dark foil, thereby stopping the access of energy. By measuring the rate of attrition of algae cells from the shaded stretch, they calculated the initial rate of growth. Similar investigations were also conducted under laboratory conditions. A chemostat was filled with sterilized or unsterilized water in which the inoculates of bacteria subject to testing were placed. Temperature and other factors were maintained at the level characteristic for the natural environment. By knowing the difference between the rate of dilution of the system and the rate of attrition of the organisms, one could calculate their rate of growth with high accuracy in the absence or presence of competing microflora in the unenriched natural water.

The most widespread and used methods of determining microbe production developed by Soviet scientists involve determining the number or activity of microorganisms in isolated water samples. Work with isolated samples of water, whose results are then extrapolated to the reservoir, forces the investigator to accept a number of assumptions. Taking the samples means moving from an open system, which the natural habitation is, to a closed system of the isolated sample, which is equal to sharply changing the environment. Another possible influence of the isolated sample on the rate of reproduction of bacteria is the effect of the walls of the vessel, since the solid surface is a point of concentration of organic material and of fixation and enhanced reproduction of the bacteria.

However, the investigations of Gak (1975) showed that the number of bacteria in unfiltered samples of water remains equal to their initial population in the reservoir for one day. This indicates that normal functioning is preserved in flasks containing water, over the course of several days at low temperature and a slow rate of processes and over the course of one day at high temperatures and a high rate of the process in the plankton society as are the natural interrelationships of the organisms, i.e., one observes neither the effect of the vessel walls nor the effect of the closed sample on the population of bacteria.

Determination of the production of bacteria in the water samples from the reservoir predicates purifying the sample of zooplankton so that their devouring the microorganisms does not blur the picture of the absolute increment of their biomass. For this purpose, filtration through gas or a filter paper is employed. Filtration disrupts the natural food relationships in the water. It has been shown that an increase in the number of bacteria is observed in the filtered samples, and in the non-filtered samples the number of bacteria remains constant for a day. However, this change in the population of bacteria in the sample as the result of filtration is a slight one and cannot influence microbial production. Obviously, however, filtration removes the epiphyte microflora attached to the algae from the sample as well as the microorganisms attached to the detritus.

Phytoplankton influences the microflora in two ways: it liberates (in life or posthumously) the organic substances dissolved in the water. Furthermore, the intensive development of microorganisms occurs on the surfaces of the old and dying algae cells; instances of a toxic or antibiotic effect of plankton algae are known.

All of the flask methods of determining microbe production include the use of dark flasks. For some reason, it is felt that this, i.e., the exclusion of photosynthesis, holds differences between the filtered and unfiltered water to a minimum. Excluding photosynthesis is vital for the method of determining production according to CO_2 fixation, so as to separate the heterotrophic and chemosynthesizing fixation from the photosynthetic variety.

It seems to us that removal of the phytoplankton during fixation has a great drawback for the goals of determining microproduction since it disrupts the process of algae liberation of organic material, and, perhaps, of toxins into the medium, the consumption by algae of the biogenic elements, the cells of algae themselves are removed, some of which serve as a direct substrate for the bacteria. All of this certainly strongly disrupts the natural process of vital activity and reproduction of the bacteria for the reservoir. Probably, this explains the unequal character of growth curves of bacteria in the isolated samples.

Hence, we conclude the limited value of any of the methods of determining microbe production. Since this determination cannot be an end in itself in the ecological research, but is only a method of identifying functioning (or potential possibilities) of the ecosystems, then certain of the listed methods can be recommended as standard ones, but under the condition of observing the combined approach based on the principles of Vinogradskiy.

In this regard we submit that it is necessary simultaneously to make the following analyses in order to judge production of microorganisms in ecosystems (with the goal of using these judgments in any ecological forecasts):

1. For representing the situation in the ecosystem:
    a. Chemical analysis for the composition of the water (the content of inorganic ions and organic material), consideration of the physicochemical conditions (temperature, illumination, pH, O_2 concentration, etc.), the determination of the makeup and number of microorganisms.
    b. Counting the total number of microorganisms by the aid of filter staining; counting the number of living cells by the aid of luminescent microscopy.
    c. Counting the groups of microorganisms that one can expect, based on the composition of the water and other conditions, quantitatively, using the method of sowing on dense elective media.

2. For determining true microbe production:

   d. Determine the total production in isolated water samples according to the method of Romanenko-Kuznetsova-Sorokin using ^{14}C according to the rate of heterotrophic, chemosynthetic and photosynthetic fixation of CO_2.

   e. Determine the potential capacity of the water to produce microorganisms by the aid of sowing various cultures in a chemostat (necessary for investigating situations with slight natural inoculation).

   f. Determine the potential activity of microorganisms in ecosystems with a natural limitation for one factor or another by means of adding and varying conditions.

USE OF BIOLOGICAL INDICATORS FOR MONITORING EFFECT OF POLLUTANTS ON THE MARINE ENVIRONMENT

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ABSTRACT

Marine organisms which exhibit sensitive physiological responses to pollutants have been used in laboratory biological assays to predict the effects of pollutants on the marine environment. The methodology for this approach is developed for single species bioassays and is under development for laboratory experimental ecosystem bioassays. The bioassay approach will serve as the primary means for predicting effects of pollutants before the pollutants are introduced to the marine environment and will be important in monitoring sublethal effects of pollutants on marine organisms.

Indicator species, opportunistic species which colonize disturbed environments, have been used for many years as indicators of pollution effects in aquatic environments. While the classical saprobic indicator system is no longer used in the United States, some groups of organisms such as Capitellid worms remain useful indicators of chronic pollution. Quantitative ecological techniques which have been developed for use in characterizing assemblages of organisms show promise for monitoring effects of pollutants. While no single measure appears to be a consistent indicator of disturbance of natural assemblages by pollutants, a combination of changes in dominance, species diversity, and evenness can be used together with ordination methods to quantitatively assess changes in assemblages of marine organisms caused by pollutant stress or by reduction of pollutant stress.

INTRODUCTION

Marine organisms which exhibit sensitive physiological responses to particular pollutants or classes of pollutants have been used in biological assays to establish permissible environmental concentrations of pollutants. This approach is the standard means by which potentially singificant pollutants are evaluated to predict their effects on marine organisms (Goldberg, 1975). Mortality of some part of the test population is commonly used as the primary means for defining pollutant effect. Additional criteria such as changes in respiration rate, inhibition of enzyme systems, and modification of blood component characteristics have been suggested for use in defining pollutant effects (Duke, 1974). Biological assay methods using single species of organisms will continue to be the primary means for predicting pollutant effects on the marine environment prior to introduction of a pollutant. Laboratory experimental ecosystem assay methods for predicting pollutant effects on marine food webs are under development and show promise. The success of both the organism bioassay methods and the laboratory experimental ecosystem assay methods for predicting potential effects of pollutants and for monitoring chronic effects of pollutants in the marine environment depends on the successful culturing of marine organisms. Many neritic phytoplankton species and some neritic invertebrate and vertebrate species presently can be maintained in the laboratory. Some neritic species can be raised through the various stages of their life cycles in the laboratory; however, it is very difficult to maintain populations of oceanic organisms in culture. Progress is being made in culturing oceanic phytoplankton (Booth, 1975). Knowledge of pollutant effects on marine organisms or marine communities is best developed for neritic environments. The investigation of pollutant effects on oceanic species is very important since oceanic species may be more susceptible to pollutants than coastal species. Diatoms isolated from the Sargasso Sea were more sensitive to polychlorinated biphenyl (PCB) compounds than were clones obtained from estuaries and from continental shelf waters (Fisher et al., 1973).

The classical saprobic system for diagnosing water pollution was devised by Kolkwitz and Marsson (1908). The system is based on zonal distribution of indicator organisms which respond to pollutants emanating from a point source. Zones of organic pollution effects and zones of recovery from pollution effects were identified based on classes or organisms present within the different zones. The saprobic system has been attacked on several bases (Sladecek, 1965) and is no longer used in the United States (Bartsch and Ingram, 1966). Marine biogeographical investigations attempt to elucidate the geographical distribution of organisms on the basis of their physiological responses to varying environmental conditions (Ekman, 1953; Hedgpeth, 1957). A central feature of marine biogeographical investigations is the identification of characteristic fauna which occupy particular water masses (McGowan, 1972) or which inhabit particular combinations of depth ranges and sediment types (Menzies et al., 1973). The combination of the biogeographical approach, with emphasis on identification of taxa inhabiting particular regions of the marine environment and with the tools of modern quantitative ecology, shows promise for assessing the effect of pollutant stresses on the biota of the marine environment. This approach may be used for evaluating the quality of predictions based on biological assay methods and for quantitatively monitoring changes in marine food web structure caused by impact of pollutants or by the reduction of pollutant impact.

BIOLOGICAL ASSAY EXPERIMENTS FOR ASSESSING POLLUTANT EFFECTS

Many potential marine pollution problems can be assessed by a detailed consideration of rates of release into the environment, residence time in the environment, concentration by marine organisms, and levels of toxicity of the compounds in question (Goldberg, 1975). The determination of toxicity levels at present is achieved primarily through biological assay experiments utilizing one or a few species of organisms maintained in the laboratory. The choice of organisms for use in bioassay experiments is constrained by the limited number of species that can be maintained in the laboratory. Experimental organisms are chosen because they are easy to handle in experimental designs, because they give particularly sensitive and reasonably reproducible responses to particular pollutants, or because they are of direct economic value. An additional criterion for choice of experimental organisms is their significance in the food webs of which they are a part. Keystone species play significant roles in controlling food web structure. A starfish, *Pisaster ochraceus*, controlled food web structure of a Washington rocky intertidal bottom through predation (Paine, 1966). Introduction of a carnivorous fish, *Cichla ocellaris*, to Lake Gatun, Panama, markedly affected the pelagic food web of the lake. Carnivorous bird numbers decreased near parts of the lake dominated by *Cichla* (Zaret and Paine, 1973). Sea otter, *Enhydra lutis*, appeared to be a keystone species in Aleutian Island and California nearshore food webs (Estes and Palmisano, 1974). Squid are hypothesized to be a pelagic oceanic keystone species which defines pelagic food web structure through predation (Costlow, 1975). To understand and to monitor effects of pollutants on oceanic food webs, research efforts must be directed toward identification of keystone species and toward elucidation of pollutant effects on these species.

Bioassay methodology has been reviewed by Sprague (1969) who recommended that the lethal concentration for 50 percent of individuals on exposure to a compound (incipient LC50) is the single most useful criterion of toxicity. The 4-day LC50 is another common measure of acute toxicity. Bioassays of synthetic pesticides indicate that toxicities of compounds cannot be predicted and that each compound must be evaluated to assess its toxicity. The effects of a pesticide on estuarine organisms cannot be predicted on the basis of its observed affinity to other known pesticides or its known action on other animal species (Butler, 1971). In an attempt to account for synergistic or antagonistic interaction, the toxicities of mixtures of two or more pollutants have been represented by a single number composed of the sum of toxicities of individual pollutants represented as fractions of the incipient LC50 (Sprague, 1970). This approach is questionable since joint effects of pollutants are not always linear (Livingston et al., 1974). Temperature, pH, bicarbonate alkalinity, salt content, totally dissolved solid content, and dissolved oxygen concentration are capable of modifying toxicities of pollutants (Sprague, 1971; Livingston et al., 1974).

It is very important that bioassay results are correctly interpreted. Most phytoplankton bioassay experiments which reported effects of pollutants on photosynthetic carbon-14 fixation appear to have estimated indirectly the effects of pollutants on phytoplankton cell division rates rather than their effects on photosynthetic rates. Photosynthetic carbon-14 uptake rates for phytoplankton exposed to PCB compounds and to DDT were not different from control values when normalized to a per cell basis (Fisher, 1975).

Pollutant effects can be more pronounced at particular stages in the life cycle of organisms. A concentration of 1.0 μg/l of the PCB Aroclor® 1254 killed juvenile pink shrimp (*Penaeus duorarum*) within 15 days in laboratory bioassay. Adult shrimp were not as susceptible to Aroclor® 1254 at low concentrations, but were killed within 17 to 53 days at 2.4 to 4.3 ppb of the Aroclor® (Nimmo et al., 1971). *Crassostrea gigas* and *C. angulata* spermatozoa survival was more sensitive to various weights of oil suspended in water than were eggs, embryos, or larvae, therefore the most pronounced effects of oil on oyster populations may occur during fertilization (Renzoni, 1973). Sheepshead minnow fry (*Cyprinodon variegatus*) were more susceptible to Aroclor® 1254 than were embryos, juveniles, and adults (Schimmel et al., 1974). Pesticide residues in water samples obtained from selected United States estuaries were low in fall and winter and rose in late spring to a peak in midsummer. This seasonal pattern probably reflected periods of pesticide application for agricultural purposes and time of maximum runoff from watersheds. Unfortunately, the seasonal maximum of pesticide residues coincides with the time when maximum numbers of larval fish and shellfish are present in estuaries (Butler, 1971).

There is growing recognition of the significance of chronic low-level pollution which does not result in spectacular point-source kills but which can modify the structure of marine food webs on a long-term basis. Sublethal effects of pesticides may be particularly important in the marine environment (Butler, 1971; Duke and Dumas, 1974). All organisms sampled from pelagic *Sargassum* weed communities in the Atlantic Ocean were contaminated with petroleum hydrocarbons (Burns and Teal, 1973). There was no relation between the hydrocarbon content and the animals' supposed positions in the food chain. There were extensive mortalities of the marine macrophytes *Fucus spiralis*, *Mya arenaria*, and *Spartina alterniflora* as a consequence of a large spill of Bunker C oil in Chedabucto Bay, Nova Scotia. Zooplankton and benthic animals apparently were not acutely affected by the hydrocarbons (Conover, 1971; Scarratt and Zitko, 1972; Thomas, 1973). Chronic effects of hydrocarbon pollution on marine communities remain unknown.

Pollutants can modify assemblages of marine organisms through nontoxic effects. DDT affected salinity selection by mosquito fish (Hansen, 1972). The grass shrimp (*Palaemonetes pugio*) avoided water containing the herbicide 2,4-D but did not avoid water containing several insecticides in laboratory experiments (Hansen et al., 1973). Fishes avoided Kraft-mill effluents in laboratory experiments (Lewis, 1974). Grass shrimp, pinfish (*Lagodon rhomboides*) and mosquito fish (*Gambusia affinis*) avoided at least one concentration of Aroclor® 1254 in water, but pink shrimp (*Penaeus duorarum*) and sheepshead minnows did not avoid water containing any of the experimental concentrations (Hansen et al., 1974).

There are some new approaches to pollutant assessment which utilize cellular or subcellular components of organisms in biological assays. Tissue culture methods can be used to monitor environmental levels of mercuric chloride which inhibited multiplication of L-cells at concentrations of 10 μg mercuric chloride per liter or less (Li and Troxlen, 1972). Enzyme assays may provide a sensitive and reproducible means for assessing effects of pollutants on marine organisms. Acetylcholinesterase activity was inhibited by organophosphate and carbamate pesticides when the fish *Leiostomus xanthus* was exposed to the compounds (Coppage, 1972). Standardized assays of enzymes of the tricarboxylic acid cycle or of the electron transport system may provide a reproducible means for estimating effects of pollutants on marine organisms (John Calder, personal communication). The use of standardized enzyme assays could minimize data interpretation problems which arise in pollutant bioassays when different populations of the same species of experimental organisms are grown under different environmental conditions. Identification of changes in tissue of marine organisms subjected to pollution stress shows promise for determining whether sublethal effects of pollutants have occurred in the marine environment. Yevich and Berry (1969) identified tumors in ovarian tissue from clams, *Mercenaria mercenaria*, obtained from Narragansett Bay, Rhode Island. Effects of various pollutants on tissue of invertebrates has been discussed by Sparks (1972).

POLLUTION INDICATOR SPECIES

The central concept which underlies the use of indicator organisms for monitoring effects of pollutants on the marine environment is that pollutant stress results in a change in species composition and in numbers of organisms. The change frequently appears to be in the direction of a simplification in community structure with equilibrium species replaced by opportunistic species (Woodwell, 1970). Since the assemblage of organisms present in a polluted area reflects the effects of pollutants over some length of time, sessile organisms or organisms with low mobility are best choices for indicator organisms. Worms, mollusks, arthropods, foraminifera, epiphytic algae and attached microalgae have been used as pollution indicators in marine environments (Wass, 1967).

Worms of the genus Capitella have been used to indicate the presence of pollutants in marine and estuarine water. Capitellid worms are found in areas polluted by sewage outfalls (Filice, 1959; Halcrow et al., 1973) and in areas polluted by industrial effluents (Wade et al., 1972; Pearson, 1972; Rosenberg, 1972). A single genotype of *Capitella capita* was selected on a short-term basis following an oil spill in a Massachusetts bay (Grassle and Grassle, 1974). In some cases, Capitellid worms have been observed to colonize areas which were physically disturbed but which were not polluted (Eagle and Rees, 1973). Apparently the cosmopolitan species *C. capita*, one of the most widely used indicator species, is actually a composite of six species which differ very little morphologically but which differ widely in life histories and in reproductive modes (Grassle and Grassle, 1976).

Highly polluted areas near a sewage outfall in Biscayne Bay, Florida, were characterized by benthic algae, *Gracilaria blodgettii* and *Agardhiella tenera*, plus a tubiculous polychaete, *Diopatra cuprea*. Less polluted areas were characterized by seagrasses, *Haladule wrightii* and *Halophila baillonis*, together with an ophiuran, *Amphioplus abditus* (McNulty, 1961). Littoral algal communities of Yugoslavian coastal waters were sensitive to organic pollution. Absence of a dominant alga, *Cystosiera barbata*, and presence of *Ulva lactuca* and *Codium tomentosum* indicated pollution effects (Golubic, 1973).

USE OF BIO-STATISTICAL MEASURES AS INDICATORS OF POLLUTANT EFFECTS

The strategy of ecological succession appears to be toward homeostasis with the physical environment in the sense of achieving maximum possible protection from environmental perturbations (Odum, 1969). The accumulation of pollutants in the biosphere has led to changes, which operate on successional time scales, in the structure and function of natural ecosystems. Patterns in the changes seem to be broadly similar for different pollutants in different ecosystems. Under pollutant stress, ecosystem structure shifts from a complex arrangement of specialized species toward species which are generalists. Species diversity, stability of nutrient cycles, and stability of population numbers appear to be reduced (Woodwell, 1970). The identification and characterization of changes that occur under pollutant stress require detailed sampling and identification of organisms in areas under pollutant stress. The large quantity of data requires condensation into biologically meaningful statistics that can be used to interpret changes in ecosystem structure due to introduction or removal of pollutant stresses. It has been apparent for some time that some waste products are subjecting marine environments, particularly nearshore ecosystems, to an increasing degree of pollutant stress. A report on "Waste Management Concepts for the Coastal Zone" (NAS-NES Committee on Oceanography, 1970) outlined some particularly important problem areas and research requirements regarding assessment of the biological status of coastal marine environments. The report emphasized the need for evaluation of various statistical indices, together with evaluation of sampling variability as it affects the indices, to determine their usefulness for assessing pollutant effects on the marine environment. The recent literature contains several examples of the use of statistical indices to evaluate pollutant effects on marine biota.

Bechtel and Copeland (1970) found lowest information theory diversity indices in the most highly polluted areas of Galveston Bay, Texas. Fish populations could be divided into separate communities, each structured as a response to environmental and pollution stress. In those areas receiving the greatest stress, the bay anchovy, *Anchoa mitchilli*, was the dominant species; the most highly stressed areas also supported the fewest number of large individuals. A four-year investigation of fish populations of the middle Patuxent Estuary in Maryland revealed strong seasonal cycles in number of species, number of individuals, and in several diversity indices (McErlean et al., 1973). Trend analysis revealed downward trends in the number of species, in species richness, in the information theory species diversity index, and in evenness. The changes were explained by loss of species and by dominance shifts, both of which could result in simpler community structure. No simple causal relationship could be established for the changes hypothesized to arise from general environmental degradation. Effects of Kraft pulp-mill effluents on the fishers of a shallow bay system along the north Florida coast were investigated for two years (Livingston, 1975). Estuarine and marsh fish assemblages in areas of acute pollutant impact contained reduced numbers of individuals and species. A survey of a broad offshore area showed reductions in number of individuals and number of species collected on a monthly basis. The cumulative number of species collected annually was the same for polluted and unpolluted areas, however. Opportunistic species, relatively rare in unpolluted areas, appeared to be recruited to areas of pollutant impact. The polluted areas showed decreased dominance and qualitative species differences compared to control areas. Species richness and species diversity (H' and the Shannon-Weaver Index, H) were lower at highly stressed stations but were similar to control values at stations where pollutant impact resulted in moderate reductions in the number of individuals and in the number of species. Species diversity by itself did not appear to be an indicator of pollutant effects for the estuarine and marsh fish assemblages. Equitability indices were the same for polluted and unpolluted areas of the shallow bay system. A general pattern of reduced numbers of individuals and species, decreased dominance, and variable species diversity and species richness were observed at most heavily impacted stations.

Macrobenthic species assemblages at stations in the Elizabeth River, Virginia, differed in terms of species content, dominant species, and species diversity when compared to stations with similar bottom types in the Hampton Roads, Virginia, area (Boesch, 1973). Species diversity, estimated with the information theory index, H', was reduced in the Elizabeth River macrobenthic assemblage. The changes in the biological indices appeared to be related to pollutant stress in the form of primary-treated domestic sewage entering the Elizabeth River. Biota around a low-volume domestic sewage outfall near San Clemente Island, California, were less diverse (in 5 diversity indices) than were controls. Several marine macrophytes were replaced by a low turf of blue-green algae, which exhibited higher net productivity, smaller growth forms, simpler and shorter life histories and which were components of earlier successional stages in the littoral zone (Littler, 1975).

Zooplankton were collected from Timbalier Bay, Louisiana, and from contiguous coastal waters to assess effects of oil extraction on the zooplankton community. No statistically significant differences in mean number of single species populations, in species diversity, or in biomass were observed for samples collected near oil platforms compared to control areas. Long-term effects of oil drilling and production on zooplankton communities appeared negligible (Marum, 1974). An investigation was conducted in the New York Bight to evaluate small-scale variations of single species populations and coastal zooplankton communities as they related to the disposal of acid waters. The spatial distribution of the majority of the species was highly aggregated, but no trends were observed to suggest that the acid wastes were an important factor in controlling the distributions (Wiebe et al., 1973). No significant trends were evident in species diversity estimated with the information theory H'. Differences of less than a factor of 5 to 10 in the abundance of a population between stations in coastal waters, based on single observations, could have been caused by sampling error. The effects of Aroclor® 1254 on survival of marine organisms was evaluated in a four-month laboratory ecosystem investigation (Hansen, 1974). Amphipods were dominant in control aquaria and in aquaria containing 0.1 $\mu g\ liter^{-1}$ of the PCB. At a concentration of 10 $\mu g\ liter^{-1}$ of the PCB, greater than 75 percent of the animals were tunicates, indicating dominance changes as PCB concentration increased. Numbers of phyla, species, and individuals in the experimental aquaria were reduced compared to control aquaria, but there were no apparent effects on abundance of annelids, brachipods, coelenterates, echinoderms or nemerteans. The species diversity (H) was not affected by this Aroclor®.

Apparently no single statistical index can completely characterize changes in community structure that occur as a consequence of pollutant stress on the marine environment. A combination of dominance measures (McNaughton, 1968), species diversity indices (Pielou, 1966), and evenness measures (Pielou, 1966), together with changes in kinds of species, can provide a basis for evaluation of effects of pollutants on marine environments. This approach is proving highly successful for evaluating changes in marine communities after cessation of pesticide and paper pulp-mill waste input to coastal waters (R. Livingston, personal communication).

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MICROORGANISMS AS BIOLOGICAL INDICATORS OF COMMERCIAL-DOMESTIC AND PETROLEUM POLLUTION

by

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Despite a large number of investigations on protecting the marine aquatoria, the intensity of autopurification, as well as the degree of chemical and commercial-domestic pollution of the northern seas, have not been practically studied.

The level of different pollutants in the water can be detected by both sanitary-chemical and sanitary-bacteriological methods. According to the observations of A. G. Mironov (1961, 1967, 1970), the sanitary-bacteriological indicators of sea water pollution are significantly more sensitive than the sanitary-chemical ones. Thus, proportional to remoteness from the source of pollution, when the oxidizability and BOD_5 of sea water already do not record pollution of the aquatorium, the latter is detected by microbiological methods which most reliably show the range of spread of pollution from its source.

The goal of the investigation is to explain the degree of pollution of two bays of the Barents Sea by commercial-domestic and petroleum pollutants according to the titer of microbiological indicators--coliform bacteria, enterococci, the petroleum oxidizing microorganisms and bacteriophages.

In all, 80 samples of water of varying degrees of pollution were investigated for the first (most polluted) bay: 40 samples of dirty water (near the coast); 25 samples of water of a moderate degree of pollution (the middle of the bay); and 15 samples of arbitrarily pure water (at the exit from the bay).

Forty-five water samples were investigated for the second bay (less polluted), 15 samples each of varying degrees of pollution. The investigations were carried out with 3 repretitions.

Data on the microbiological indicators of pollution of the bays are given in Tables 1 and 2.

It is apparent from Tables 1 and 2 that the dirtier the water, the lower the titer of the sanitary-indicative microorganisms; in this instance, a change in the titer of indicators of the commercial-domestic pollutants correlated with the titer of the petroleum-destroying microorganisms.

TABLE 1. TITER OF INDICATOR MICROORGANISMS IN WATER SAMPLES OF THE FIRST BAY

Type of microbe	Season	Degree of water pollution		
		Dirty	Moderately polluted	Arbitrarily clean
Coliform bacteria	Summer	$10^{-5}-10^{-6}$	$10^{-2}-10^{-5}$	$1.0-10^{-1}$
	Winter	$10^{-3}-10^{-4}$	$10^{-1}-10^{-2}$	1.0
Enterococci	Summer	$10^{-4}-10^{-5}$	$10^{-1}-10^{-2}$	$1.0-10^{-1}$
	Winter	$10^{-2}-10^{-3}$	$10.0-10^{-1}$	1.0-10.0
Petroleum oxidizing microorganisms	Summer	$10^{-3}-10^{-4}$	$10^{-1}-10^{-2}$	1.0
	Winter	$10^{-3}-10^{-3}$	10^{-1}	Over 1.0
Total microbe number in thousands of microbial bodies per ml	Summer	80.0	4.0	0.8
	Winter	45.0	2.5	8.2

TABLE 2. TITER OF INDICATOR MICROORGANISMS IN WATER SAMPLES OF THE SECOND BAY

Type of microbe	Season	Degree of water pollution		
		Dirty	Moderately polluted	Arbitrarily clean
Coliform bacteria	Fall	$10^{-1}-10^{-2}$	$1.0-10^{-1}$	1.0-10.0
	Winter	10^{-2}	$1.0-10^{-1}$	10.0
Enterococci	Fall	$1.0-10^{-1}$	10.0-1.0	10.0
	Winter	10^{-1}	10.0	10.0-100.0
Petroleum oxidizing microorganisms	Fall	10^{-2}	10^{-1}	1.0-10.0
	Winter not determined			
Total microbial number in thousands of microbe bodies per ml	Fall	6.0	0.7	0.1
	Winter	9.0	1.5	0.2

In the first bay, investigations were carried out to identify the relationship of the percentage of intestinal phages and the degree of pollution of the aquatorium with commercial-domestic organic material. A total of 135 water samples was investigated (45 samples each from points with different degrees of pollution).

The following cultures were employed for phage nutrients: *Escherichia* *coli*, *Shigella sonnei*, *Shigella flexneri*, *Salmonella newland*, and *Salmonella breslau*. The frequency of isolating phages from the water samples of different degrees of pollution is given in Table 3.

TABLE 3. FREQUENCY OF ISOLATING PHAGES FROM SAMPLES OF BAY WATER (in %)

Test microbes	Without inoculation			With inoculation		
	1	2	3	1	2	3
Escherichia coli	13.3	0	0	55.5	26.6	11.1
Shigella flexneri	2.2	0	0	31.1	15.5	4.4
Shigella sonnei	2.2	0	0	22.2	6.6	0
Salmonella newland	0	0	0	11.1	0	0
Salmonella breslau	0	0	0	4.4	0	0

Note: 1--dirty water; 2--moderately polluted water; 3--arbitrarily pure water

From the analysis of the table, one can see the clear relationship of the percentage of isolation of phages and the degree of purity of the water.

Without inoculation (which indicates the high concentration of phage-sensitive bacteria), only phages to *E. coli*, *Shigella flexneri*, and *Shigella sonnei* were isolated from samples of dirty water with a titer, according to Appel'man, of 10^{-2}.

With inoculation, phages were isolated from all three categories of water samples, and the greatest percentage of isolation of phages fell to the phages to *E. coli* and *Shigella flexneri*.

Phages of moderate and strongly lytic activity to coliform bacteria and the Shigellas (10^{-5}-10^{-8}) and low lytic activity to the Salmonellas (10^{-2}-10^{-3}) were isolated from dirty water samples.

A study of the range of valents and specificity of the isolated phages showed that the spectrum of action of phages within the confines of its species is very high. Thus the bacteriophage of *E. coli* lysed 100% of the test cultures (40 strains).

Most phages were strictly species specific, and only three varieties of the phage of *Shigella sonnei* lysed *Shigella flexneri*. In most of the phages the colonies were circular. The dimensions of the negative spots strongly varied from very small (0.1 x 0.1) to large (5 x 5 mm). In some of the phages, the formation of sterile spots of various size and morphology was simultaneously observed (transparent colonies, transparent colonies with a dark center, dark colonies with a transparent center), which indicates the multiplicity of phage types to the sensitive bacteria.

As the result of the investigations, a parallel was established between finding phages in the water, the coli-titer, the titer of enterococci and the total microbe inoculation.

Hydromechanical (or physico-oceanographic) factors influenced the degree of pollution and the intensity of processes of autopurification of marine aquatoria to a significant degree. In the second bay the authors followed a change in the titer of the sanitary-bacteriological and petroleum-oxidizing microorganisms in connection with the tidal hydrological regime of the aquatorium.

The titer of the indicator microorganisms was determined during maximum high and low tides in the southern part of the bay, where waste waters are continuously discharged, and in the northern part of the bay which washes through the sound to the open sea.

The relationship of titers of the indicator bacterioflora and the hydrological regime of the bay is shown in Table 4 (an average of 6 repetitions is given).

TABLE 4. THE EFFECT OF HIGH AND LOW TIDES ON THE TITER OF THE SANITARY-INDICATOR AND PETROLEUM OXIDIZING MICROORGANISMS OF THE MARINE BAY

Type of microbe	At outlet to the open sea		At discharge point of waste water	
	High tide	Low tide	High tide	Low tide
Petroleum oxidizing microorganisms	0.1	0.01	0.01	0.001
Coliform bacteria	10.0	1.0	0.01	0.001
Enterococci	1000.0	10.0	1.0	0.1
Total microbe number in thousands of microbe bodies per ml	0.1	0.5	4.0	15.0

It is apparent from Table 4 that the fluctuation in the titer of the indicator microorganisms during high and low tides indicates the significant role of the tidal hydrological regime in the autopurification of the studied aquatorium from the commercial-domestic and petroleum pollutants.

A study was also made of the distribution of petroleum-oxidizing microorganisms in open regions of the Barents Sea, which is less polluted with petroleum products than the bays. Sampling was carried out at 141 stations. In the coastal zones of the sea a number of petroleum oxidizing microorganisms fluctuated from 1 to 30 specimens per ml, and in the open regions, up to one specimen per 100 ml.

CONCLUSIONS

1. The systematic detection of phages of the coliform group in samples of polluted water indicates the indicator role of the given microorganisms.

2. The titer of petroleum oxidizing microorganisms in the aqueous environment can serve as a criterion of pollution of the studied aquatorium from petroleum pollutants.

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PERSISTENCE LIMITS IN ECOLOGICAL SYSTEMS

by

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ABSTRACT

Establishment of persistence limits based upon previous theoretical, field, and laboratory studies was found to be either inappropriate or inconclusive. Laboratory simulations appear to be the most promising approach to the approximation of these limits.

THE PROBLEM

Large-scale ecological systems have been and are being subjected to various disturbances (e.g., hurricanes, forest fires, glaciation). Over geologic time, adaptation to these disturbances has resulted in the present-day systems. However, within the last two centuries some disturbances, such as nutrient and metal inputs, have proceeded at rates greater than those observed in the past (Anonymous, 1975; Goldberg et al., 1976; Elias et al., 1975). Also, natural systems have never experienced some types of disturbances, such as radionuclides and polychlorinated biphenyl, at present levels and/or rates of addition. Much attention has been given to the temporal description of systems continuously disturbed by anthropogenic inputs (e.g., Menzel, personal communication; Hall et al., 1970; Odum and Chestnut, 1970; Schindler et al., 1973; Woodwell, 1962, 1970). While informative, studies of this kind indicate only what kind of system changes have occurred; they do not, however, address the fundamental question of the persistence of the system in the face of such disturbances. The objective of this paper is to define persistence limits and identify an experimental framework suitable for the measurement of such limits based on previous studies.

PERSISTENCE LIMITS

As with many ecological terms, persistence has various definitions. For Holling (1973), a disturbed system persists as long as the *interrelationships* between the state variables (e.g., species) are maintained: permanent changes in structure but not function are allowed. In the present communique, a disturbed system persists, provided it will revert both structurally and functionally to its predisturbed state once the disturbance is relaxed. A *persistence limit* of a system is defined as that disturbed state beyond which the system is irreversible. Each unique disturbance maps a persistence limit; the family of such limits defines the persistence limits of a system. From a theoretical and management point of view, the latter definition of persistence and the identification of persistence limits are considered more important than Holling's since the system is maintained in its totality. If the changes in system-state during anthropogenic disturbances are confined within the persistence limits, then the system or resource will be maintained by definition. Thus, persistence limits become a logical management tool.

There are a number of prerequisites to and constraints on the above operational definition of persistence and persistence limits. First, the unperturbed system of interest must be adequately defined in time and space and has holistic properties. Multiple stable points (Sutherland, 1975) are also inherent in the unperturbed system. Second, the time frame for recovery must be short relative to possible long-term (1000 years depending upon the generation time of the slowest biotic element) natural changes in equilibrium states (see Botkin and Sobel, 1975). Third, the ability to determine a change in the system during a disturbance will be technology dependent (Woodwell, 1975). A change in a system exceeding a persistence limit could occur but remain undetected because of the lack of sensitivity in the prevailing technology. However, it is possible to indirectly detect the presence or absence of thresholds of systems response by regression techniques applied to disturbance response data at detectable levels.

PERSISTENCE LIMITS AND PREVIOUS STUDIES

A. THEORETICAL STUDIES

Computer simulation and analytical models of ecosystems abound (May, 1975; Patten, 1971). Measures of persistence limits for these models might provide estimates for natural systems. The validity of such estimates assumes the models are adequate representations of the field or total system. However, the data base and principles from which most complex models are formulated are usually derived from isolated components of a system. These components are experimentally isolated from the total system before experiments are performed. If systems are holistic (Gallopin, 1971), then even a detailed knowledge of their parts will not provide the information necessary to describe the total system. Walters and Efford (1972) showed that a complex model derived from a ten-year study of isolated components of a lake ecosystem provided limited dynamical information. Therefore, the establishment of persistence limits using previous theoretical models is inappropriate.

B. FIELD STUDIES

Experimental studies in the field dealing with the recovery of disturbed, complex systems are few in number and inconclusive. It is difficult in terms of logistics, cost, and time to disturb and observe the recovery of large-scale ecosystems. Adequate controls (unperturbed replicates) often do not exist or are unavailable. Cause-and-effect relationships in the field are sometimes difficult to interpret because of unknown and/or uncontrollable disturbances. These difficulties may explain the paucity of large-scale field studies. The few studies which deal with recovery usually suffer from constraints of time, scale, and control. For example, Bormann et al. (1974) demonstrated the recovery after three years of two functional system properties, the fluxes of dissolved and particulate material after cutting the large primary producers and applying chemical defoliants to a deciduous forest. However, complete structural recovery (if it ever occurs) will take half a century or more. Similarly, following fumigation with methyl bromide, Simberloff and Wilson (1970) showed after two years the incomplete recovery of the terrestrial anthropod species found on mangrove islands. After almost thirty years, sewerage import into Lake Washington ceased. However, four years later, the relaxation of the sewerage has not resulted in the complete recovery of the zoo-phytoplankton structure although concomitant watershed changes may confound this recovery (Edmondson, 1972).

Through removal of a predatory starfish, *Pisaster*, from an intertidal community for five years, a predation disturbance resulted in a shift in the age structure of its prey, *Mytilus* (Paine, 1974). Even after re-exposure to starfish for seven years, the older and larger *Mytilus* remained (Paine, personal communication). The starfish, perhaps because of energy constraints, did not attack these larger *Mytilus*. Whether this new structure will provide a refugium for newly settling *Mytilus* in the future and, thus, propagate this structure is unknown. This study possibly demonstrates the existence of a system-state exceeding a persistence limit.

Qualitative and quantitative temporal characteristics of a disturbance push a system toward its persistence limits. In Paine's (1974) study, the essential characteristic was the duration of the predation disturbance. If the starfish were excluded for a specific time interval less than five years, then the resulting smaller increase in the age structure of *Mytilus* would be reversed following starfish re-entry. How critical other temporal properties of a disturbance, such as intensity, pattern (deterministic-stochastic), speed and acceleratory rates, are to the displacement of a system beyond its persistence limits has yet to be described.

C. LABORATORY STUDIES

Because of the difficulties inherent in field studies, many ecologists have attempted to develop laboratory microcosms as analogues of large-scale systems. However, before attempting to measure persistence limits in microcosms, it is necessary to know how accurately (if at all) the laboratory system approximates the natural system. If one could identify properties that define a system, then the criteria for testing the adequacy of laboratory simulation would follow. However, because each investigator has differing views concerning those properties, the criteria become dependent upon the investigator. For example, Paine (personal communication) feels that microcosm studies excluding large macrofauna are questionable. Alternately, Gordon et al. (1969) concluded that their microcosms were analogues of a natural system because *some* generalized system properties were observed. However, it should be noted that as the criteria for adequate simulation become more generalized, a false conclusion that the laboratory system mimics the natural system becomes more likely. The importance of macrofauna, turbulence, water turnover and gas exchange, spatial and temporal heterogeneity, etc., to the simulation of natural systems in the laboratory remains to be determined. Comparisons, where possible, of the microcosms and in-situ natural systems being simulated are first steps to field confirmation of laboratory systems. Perez et al. (in preparation) have compared various structural (species lists, nutrients, chlorophyll, and ATP) and functional (benthic and pelagic metabolism and nutrient fluxes) properties of laboratory microcosms to the field and found it possible to accurately simulate a complex marine system in the laboratory for more than half a year. The measure of persistence limits on these laboratory systems will provide estimates of the natural system. No one has attempted to establish persistence limits for complex microcosms (Levandowsky, in press).

It is my view that the microcosm approach, with adequate experimental and field control, is the most promising vehicle for providing approximations of persistence limits of large-scale ecological systems.

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THE DEVELOPMENT OF STANDARD LABORATORY AND FIELD ECOSYSTEM RESEARCH TO DETERMINE THE EFFECTS OF POLLUTANTS ON THE MARINE ENVIRONMENT

by

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For the sake of convenience in examining the problem of the specifics of research into the effects of pollutants on the marine environment in laboratory and field conditions, it is expedient to study the concepts of the organizational levels of living organisms currently existing in published material. Such an approach made it possible for V. D. Fedorov (1974) to develop fundamental principles of diagnostic and prognostic monitoring. In developing these principles, we have first undertaken to systematize some of the biological parameters (variables) described in the literature (the "biological response," in Fedorov's terminology) which lend themselves to measurement.

Many efforts to classify the structural and functional parameters on the basis of the organizational levels of living organisms have heretofore been unsuccessful for a variety of reasons. Primary among them has been a lack of precision in the concept of correspondence between certain "biological responses" and the levels of organization. By way of overcoming these obstacles, we have once again undertaken to compare two systems of the levels of organizing living organisms existing in the literature. According to the Odum system (1975), the spectrum of organizational levels is characterized as a horizontal series so that, in his opinion, all levels deserve the attention of researchers to the same extent. And, with a shift to the right from genetic systems to ecosystems, some parameters become more important and more changeable, while the importance of others becomes negligibly small and their changeability is hardly notable. The picture will appear somewhat different if the diagram of organizational levels suggested by N. P. Naumov (1972) is used as the basis for study.

With such an approach, it is possible to isolate the characteristic level of the structural hierarchy of the organic world in which the integration of parts reaches such a degree that the system is capable of isolated existence and independent reproduction of the system, and those relative to the lower and intermediate levels do not possess such capabilities.

Therefore, it would be justified to examine the structural and functional parameters of the upper but not those of the intermediate and lower levels. A collection of biological responses for the lower and intermediate levels of the molecular-cellular and organismic stages would depend primarily on the developmental level of the methodical concepts derived from physics, chemistry and cybernetics primarily in molecular biology, biochemistry and biophysics. An extensive arsenal of methodical concepts of molecular biology and biophysics enables us to obtain varied information such as the kinetics of intercellular processes and the structure of vitally important macromolecules, biological membranes or cellular organoids.

TABLE 1. HIERARCHY OF THE STRUCTURE OF THE ORGANIC WORLD

Levels	Stages		
	Molecular-cellular	Organismic	Superorganismic
Lower	Molecules of one class	Tissues	Populations, species
Intermediate	Cellular organoids	Organs, their systems	Biocenotic complexes
Higher	Cells	Organisms	Biocenoses, biomes

The theoretical number of morphological and physiological parameters of an organism is infinite (Serebrovskiy, 1973). In each instance, the quantitative measure of the biological response of a morphological and physiological parameter will depend on the methodical possibilities. In resolving practical problems in the fields of medicine and applied biology, those methodical concepts which can be used without particular complications for the measurement of biological responses--and having a high correlation with such important parameters as mortality or viability, reproductivity and the continuity of life, and so on--will be pressed into service first.

Mechanisms underlying the basis for the dysfunction of various cells, organisms, whole populations or communities as biological responses to the effect of one or more harmful factors will be distinguished from one another, if only because of the various collections of systemic parameters inherent in each state of a multilevel hierarchy of the structure of living organisms.

It is especially important to bear in mind the considerations outlined above in setting up standard programs of laboratory and ecosystem field research to determine the effects of pollutants on the marine environment. The employment of concepts pertaining to the organizational levels of living organisms allows us to make more precise the sphere of applicability of experimental results obtained in the laboratory and the potential for using them to explain the functioning processes of superorganismic systems. The rules which should be used in structuring programs of laboratory and field ecosystem research are presented below.

Rule I. Laboratory research data on the condition of cells and organisms from which the entire ecosystem is made up cannot be used unequivocally to describe the condition of the superorganismic level.

Rule II. A parametric description of the status of the superorganismic levels of organization (population, communities, ecosystem) does not necessarily include the parametric description of all its constituent elements.

Rule III. Each level is described with a finite number of systemic parameters whose measurement is interpreted in accord with a generally accepted standard method.

Rule IV. Standard programs of laboratory research include a mandatory collection of test organisms cultured under standard conditions and a detailed description of standard toxicological methods.

Rule V. At a minimum, standard programs of laboratory research include experiments on various species of test organisms, on populations and simulated ecosystems (microcosms).

Rule VI. Standard programs of field research, in addition to measuring systemic parameters for the appropriate level of the superorganismic stage, should also include the formulation of active experiments based on plans provided that the gradients of pollution distribution in the aquatic environment are known.

Rule VII. The conduct of laboratory experiments is warranted only in cases where the level of aquatic pollution being studied is known and the consequences brought about by its action cannot be established on the basis of the ecosystem parameters.

THE FEASIBILITY OF DEVELOPING "STANDARD" LABORATORY STUDIES TO DETERMINE THE EFFECTS OF POLLUTANTS ON THE MARINE ENVIRONMENT

In order to evaluate the effect of pollutants on various species and communities inhabiting the open ocean on a quantitative basis, it is necessary to conduct long-term field observations. However, this is not always possible because of the problems associated with culture in laboratory conditions. In this case, the only departure from the established position will be the conduct of experiments in other sensitive species of marine organisms which can be easily cultivated in laboratory conditions. Thus, by sensitive organisms we mean organisms which are quite sensitive to any sort of pollutant; for example, crustaceans react to parts per million of chlorinated hydrocarbons and certain metals. They can be considered sensitive "indicator" organisms to these pollutants.

Laboratory research on various species of the organism should be primarily devoted to determining: 1) the rate of accumulation and rate of removal of the pollutant; 2) the relationship between the pollutant concentration in the organism and its concentration in the water; 3) the permissible concentration according to generally accepted methods; 4) the remote consequences of small concentrations; 5) the effects of endangering organisms at the molecular level; and 6) the mechanisms of the toxic effect of pollutants.

STUDYING THE EFFECTS OF POLLUTANTS IN LABORATORY MICROCOSMS

Microcosms are already extensively used to solve the theoretical and practical problems of ecology. The fact that a large portion of the work is performed in fresh water microcosms notwithstanding, there are no major obstacles in using laboratory microcosms with salt water and marine organisms. In view of the low density of populations of pelagic plankton and fish communities in the open sea, it is difficult to evaluate the effect of pollutants on their structure and function. A departure from the established position can be the staging of experiments on microcosms established on small islands, in inlets (on floating rafts) or on ships. It is possible to use such parameters as growth, reproductivity, physiological process and the variety of species as criteria for the biological response to pollutant action in such laboratory microcosms. The simplest models of microcosms must meet certain requirements even though it is virtually impossible to suggest a universal microcosm model. In biology and in medicine, experimentors use white strainless mice or some strains of pure lines as experimental "living test tubes." The universality of a utilized object certainly serves as a basis for juxtaposition of the results obtained.

At present it is possible to observe an analogous situation with only one of the limits of hydrobiology, water toxicology, where monotypical organisms are rather extensively used in biological experimentation in standard conditions. In the ecological literature, we did not encounter any works where an experiment would have created a universal model of a microecosystem or the figurative "ecological mouse." It is theoretically possible to take two independent courses in creating an "ecological mouse." One is to "carve out" a portion of the natural ecosystem and integrate it into the conditions of laboratory containers, while continuing to imitate natural changes in the basic physicochemical parameters of the environment while maintaining consistency in others. In this instance, it is possible to transfer the results obtained in a laboratory experiment to the natural ecosystem with some degree of accuracy as is done in many cases where the results of experiments carried out on laboratory mice are projected onto higher mammals.

Another method of creating an "ecological mouse" begins with the selection of organisms found in definite feeding relationships and capable of normal life activity in laboratory conditions. A system consisting of a fish (*Gambusia affinis*), a crustacean (*Daphnia magna*), a snail and algae (Isensee et al., 1973) can serve as a typical example of a microecosystem. This type of microcosm can be used to study the movement of pollutants resistant to biological degrading throughout the entire food chain. The importance of establishing such experiments lies in the fact that each of the constituent organisms can serve as a test organism in toxicological experiments, so that data obtained in various cultures will have an unquestioned value when superimposed on data obtained in experiments on entire microcosms. Experiments with long-lived pollutants introduced into the surrounding environment in relatively small concentrations (mercury, cadmium, and DDT, for example) have a special importance in this type of experiment.

Establishing such experiments on the basis of standard algorithms allows us to make more precise the sphere of applicability of concepts pertaining to permissible concentrations, with the potential for expansion to the concept of an ecological norm of pollution.

STANDARDIZATION OF FIELD ECOSYSTEM RESEARCH TO DETERMINE THE EFFECTS OF POLLUTANTS ON MARINE ECOSYSTEMS

At the present time, there is an extensive network of field research programs on the world's oceans at national and international levels. Basically, specialized scientific-research vessels and, in some cases, ships conducting nonsystematic studies whose primary task is the performance of commercial, transport and other types of work take part in research programs.

Field ecosystem studies to determine the effects of pollutants on marine ecosystems are, in very rare instances, set up in accord with an extensive program involving several years' observation of the condition of the biotic component. In our view, the main reason for this is the absence of precise concepts relating to systemic indicators characterizing the integral properties of ecosystems. At best, field studies attempt to include a determination of the majority of known physicochemical and biological indicators which can be potentially useful in evaluating the effect of pollutants on marine ecosystems. In terms of form, field studies will be only slightly different from programs of field research into fresh water ecosystems. And so it goes. The accumulation of data obtained in field studies does not result in qualitative progress for evaluating the status of marine ecosystems.

The conduct of field studies should be put into practice by standard methods proceeding, first of all, from systemic indicators leading to characterization of the integral properties of the superorganismic stage of the structural hierarchy of the organic world.

Standardization at a given stage should mean the isolation of a framework of systemic indicators which can be used to evaluate the effect of pollutants on the marine ecosystem. Beginning with a minimum framework of systemic indicators, the unification and intercalibration of determinative methods should be accomplished during the second stage of field study standardization. At the third stage, all procedures for the collection of data, its analysis and storage as well as procedures for retrieval and exploitation should be unified. At this point, the work should conclude with the development of standard documentation starting with the collection of field data and ending with storage.

THE COMBINATION OF LABORATORY AND FIELD RESEARCH IN ORGANIZING BIOLOGICAL MONITORING OF THE EFFECT OF POLLUTION ON THE MARINE ENVIRONMENT

The presently existing skepticism regarding the extrapolation of results obtained in experiments with various test organisms on entire ecosystems is fully justified for the reasons discussed above. There are some means for overcoming these obstacles, but all of them are associated with significant material expenditures. It is precisely for this reason that many proposed projects have not been successfully realized.

While not rejecting the means or methods of combining laboratory and field research established by tradition in each nation or scientific collective, it is suggested that some of the obvious approaches be shifted from laboratory experiment to field studies. This would enable us to see the degree of similarity and the possibilities for extrapolating laboratory data onto a situation in natural conditions.

From the functional point of view, it is possible to study an ecosystem to some degree from various aspects in relation to the stated question and the types of specialists and equipment. An ecosystem can be characterized by flows of energy (inward and outward), by the complexity of the food chain, the diversity of organisms and the cycle of matter. It is obvious that the action of any disruptive factor, including pollution, can substantially change one of the functional characteristics of the ecosystem. In natural ecosystems, this can be established on the basis of lengthy observations over several years within the conditions of previously planned water areas, if a possible connection between the level of pollution and the change in the amplitude of one of the functional characteristics of the ecosystem can be found.

In the overwhelming majority of cases, field ecosystem research is carried out on a case-to-case basis. In these conditions, the presence of data on the pollution level in the water areas of interest enables us to propose a number of standard laboratory ecosystem studies.

MICROCOSM WITH A VOLUME OF 1-5 LITERS

1. A microecosystem consisting of one or several populations and placed in rigidly controlled conditions, for example, in the conditions of the conventional chemostat (Carpenter, 1969).

2. Microecosystems consisting of several pure population cultures with a closed cycle of food substances (Nixon, 1969).

3. Microecosystems consisting of a small portion of a natural ecosystem obtained through multiple reseedings (Beyers, 1963).

4. A microcosm consisting of the minimum collection of food chain organisms (including fish) assuring a closed cycle of food substances (Isensee et al., 1973).

5. A microcosm consisting of species or groups of organisms important from the point of view of the simulated ecosystem living in natural conditions (Strickland, 1967).

6. A microcosm consisting of abiotic and biotic components combined in a reservoir or basin populated with basic or ecologically important organisms or groups of organisms.

7. A microcosm simulating as far as possible the factors of the external environment in laboratory conditions (the combination of species, temperature, illumination, dissolved oxygen, bottom contours), basic biotic

components from the point of view of trophics and the rate of introducing pollutants into the microcosm (Falco and Sanders, 1973).

The listed variants of the microcosm can be prepared in laboratory conditions. In order to carry out studies on these microcosms, the following is required: a) well-equipped aquatic basins and ponds as experimental bases; b) experimental basins or continuous-flow canals with an established hydrologic regime (simulated water currents, micromodels of reservoirs, and so forth); c) automated means for observing, collecting and analyzing the information obtained from experimental objects; and d) analog machines and third generation data processing machinery.

The second direction of research is strongly associated with the conduct of ecosystem research in field conditions; however, the methods will differ slightly from those employed in the study of laboratory microcosms. Microcosm size in field conditions will depend primarily on the problem to be solved and technical and financial capabilities. In all, we propose to distinguish three types of microcosm variants:

1. The small microcosm with a capacity not exceeding 50 liters enables us to isolate a small portion of the natural ecosystem with a transparent membrane (plastic being used most frequently) and to carry out observations based on a pre-planned program (Fedorov, 1974).
2. The intermediate sized microcosm with a volume of more than 50 liters isolates a portion of a natural ecosystem into which not all trophics are introduced (Davis, 1973).
3. The large microcosm enables us to study an isolated ecosystem in all its complexity (Chem. Eng. News, 1973).

Conducting research in isolated microcosms in field conditions has a tremendous advantage over laboratory studies. The main advantage lies in the fact that the isolated microecosystem being observed is under the influence of the same external environmental factors as the entire ecosystem. The introduction of pollutants into an isolated microcosm makes it possible to predict the fate of the entire ecosystem with the highest degree of accuracy.

In this way, ecosystem research on laboratory microcosms provides a clarification of the general principles of microcosmic response reactions to different influences including pollutants of various sorts. Laboratory models of microcosms, as a rule, remain remote copies of natural ecosystems. Therefore, the results of experiments performed on them can be extrapolated to natural ecosystems with such a degree of accuracy that all of their component parts and the connection between them has been taken into consideration. The latter requires knowledge of the structure of natural ecosystems and its functional specifics.

Together with the exploitation of the field variants of microcosms, ecosystem studies enable us to obtain results which can be extrapolated with complete validity to entire ecosystems, particularly in cases where they are applied to intermediate and large microcosms.

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IMPACT OF PESTICIDES ON THE MARINE ENVIRONMENT[1]

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ABSTRACT

The impact of pesticides on the marine environment can be assessed by monitoring their occurrence in the marine environment and by evaluating their toxic effects in laboratory bioassays. Acute static and flow-through bioassays generally have been used to set marine water quality criteria, but bioassay techniques now can determine effects of long-term exposure to one or more toxicants on survival, growth, and reproduction of individual species of mollusks, arthropods and fishes and effects on communities of estuarine organisms in the laboratory. Bioassays have been lengthened from 96 hours or less to between one month and two years, and their complexity has also been broadened. Effects of toxicants on the entire life cycle of an oviparous estuarine fish, *Cyprinodon variegatus*, can now be studied, and bioassays have been completed with endrin and heptachlor. Preliminary experiments using this fish revealed that they typically develop from an embryo to maturity in 10 to 14 weeks, with about 70% survival in the laboratory. Females produce an average of eight eggs per day and fertilization success exceeds 90%. Effects of a polychlorinated biphenyl, Aroclor® 1254, and of a pesticide, toxaphene, on developing communities of estuarine animals have been investigated. These studies provided data for predicting pollution-induced shifts in composition of estuarine animal communities.

INTRODUCTION

Pesticides occur in biological and physical components of coastal and oceanic ecosystems. Some have been implicated in degradation of portions of the environment because pesticides either adversely affected organisms or were bioaccumulated in organisms to concentrations deemed excessive for human consumption. However, data on direct toxic effects of pesticides on marine organisms are limited. Effects induced during chronic pollution are difficult to observe and, if observed, only rarely provide data sufficient to implicate a pesticide directly. Consequently, potential impacts of pesticides and other pollutants must be assessed through laboratory studies. Laboratory bioassay data, when properly evaluated, can aid in predicting effects in the field (Mount, 1974).

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[1] Contribution No. 279, Environmental Research Laboratory, Gulf Breeze.

The bioassay is perhaps the most useful technique available to the biologist for predicting the potential hazard of a chemical. It can vary considerably in complexity and utility, and each procedure has advantages and disadvantages. These tests may be relatively simple acute static and flow-through bioassays or complex chronic entire life cycle and community bioassays. Flow-through bioassays usually measure acute stress with more sensitivity than static bioassays, whereas life cycle and community bioassays provide better estimates of "safe" concentrations from which water quality criteria can be derived.

This paper describes bioassays conducted at the Environmental Research Laboratory at Gulf Breeze, Florida, to test effects of toxicants on estuarine animals. These bioassays include: (1) acute bioassays conducted at constant salinity and temperature and include measurements of concentration of the toxicant in water and test organism and statistical analysis of mortality data; (2) bioassays on sensitive larval stages of crabs and shrimp; (3) bioassays over the reproductive portion of, or the entire life cycle of grass shrimp (*Palaemonetes pugio*) and sheepshead minnow (*Cyprinodon variegatus*); and (4) bioassays on communities of benthic macroinvertebrates.

BIOASSAY TECHNIQUES

A. ACUTE BIOASSAYS

Acute toxicity experiments are usually conducted to determine the quantity of chemical that will adversely affect a certain percentage of the test organisms in a short period of time. The data are used to compare relative toxicity and relative sensitivity. Comparisons are most reliable when bioassay methods are uniform and when the tests produce valid statistical data supported by chemical analyses of test water.

Acute flow-through bioassays have been conducted on some pesticides to provide 96-hour LC50's (concentration lethal to 50 percent of the animals) supported by statistical and chemical analyses. Results of recent experiments show that penaeid shrimp are usually more sensitive to chemicals than are oysters, grass shrimp or estuarine fishes (Table 1). Acute toxicities of tested chemicals, except methoxychlor, exceeded those of acute bioassays published in the Blue Book (NAS-NAE Committee on Water Quality Criteria, 1972).

Acute bioassays have used flowing water of constant temperature and salinity to improve comparisons of results. Bioassays (Table 1) of DDT, heptachlor (99%), heptachlor epoxide, lindane and methoxychlor were conducted at 25°C and 20 ppt salinity. The salinity was controlled by an inexpensive device in which appropriate amounts of fresh and saltwater were added through solenoid valves controlled electrically by a photocell that perceives changes in water density indicated by a floating hydrometer (Bahner and Nimmo, 1975a). This device has been used successfully for periods of up to 9 months to maintain constant (±1 ppt) salinity during bioassays.

TABLE 1. NINETY-SIX HOUR LC50's AND 95% CONFIDENCE INTERVALS FOR THE SPECIES OF ESTUARINE ORGANISM MOST SENSITIVE TO SELECTED ORGANIC CHEMICALS IN FLOW-THROUGH BIOASSAYS. USUALLY, THE AMERICAN OYSTER, TWO FISHES AND TWO ARTHROPODS WERE TESTED. CONCENTRATIONS IN WATER WERE MEASURED BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY.

Chemical	Sensitive Species	96-hour LC50 (μg/l)	Reference
Chlordane	Pink shrimp	0.4(0.3-0.6)	Parrish et al., 1976
DDT*	Brown shrimp	0.1(0.1-0.2)	Schimmel and Patrick, unpublished**
Dieldrin	Pink shrimp	0.7(0.4-1.2)	Parrish et al., 1973
Endrin	Pink shrimp	0.04(0.02-0.05)	Schimmel et al., 1975
HCB	Pink shrimp	>25	Parrish et al., 1975
Heptachlor (74%)	Pink shrimp	0.1(0.07-0.1)	Schimmel et al., 1976
Heptachlor (99%)*	Pink shrimp	0.03(0.02-0.04)	Schimmel et al., 1976
Heptachlor Epoxide*	Pink shrimp	0.04(0.001-0.1)	Schimmel et al., 1976
Lindane*	Pink shrimp	0.2(0.1-0.2)	Schimmel and Patrick, unpublished**
Methoxychlor*	Pink shrimp	3.5(2.8-4.4)	Bahner and Nimmo, 1975b
Toxaphene	Pinfish	0.6(0.5-0.7)	Schimmel et al., in press

*Less than five species of estuarine animals tested.

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B. SENSITIVE LIFE STAGE AND LIFE-CYCLE BIOASSAYS

Long-term bioassays on sensitive life stages and on life cycles of estuarine organisms are usually conducted to determine the quantity of chemical that can be tolerated by a species throughout its life cycle or during a critical portion of its life cycle. Data from this type of bioassay are especially important in deriving water quality criteria. Water quality criteria are used to protect that species and, it is hoped, the ecosystem from chronic effects of a pollutant. Water quality criteria are frequently obtained by multiplying the 96-hour LC50 of the most sensitive species tested by an arbitrary application factor. The arbitrary application factor for persistent pollutants is usually about 0.01 (NAS-NAE Committee on Water Quality Criteria, 1972). Scientifically derived application factors can be obtained by comparing data from acute bioassays with bioassays in which a fish or invertebrate is exposed to the chemical throughout its life cycle. The factor is obtained by dividing the concentration of a toxicant that does not affect survival, growth or reproduction of a species in life-cycle bioassays by the chemical's 96-hour LC50 for that species (Mount and Stephan, 1967; Eaton, 1973).

Sensitive Life Stage Bioassays

Marine toxicologists have not been able to derive experimental application factors based on life-cycle exposure because techniques for maintaining marine cultures were lacking. Therefore, it is necessary to develop and to use methods that provide toxicity data on sensitive stages of the life cycle of saltwater species. Our laboratory funded grants and contracts to investigate effects of pesticides on larval development of dungeness crab, *Cancer magister*; blue crab, *Callinectes sapidus*; and the xanthid crab, *Rhithropanopeus harrisii*. Current or past investigations used captan, carbofuran, chlordane, DEF, malathion, methoxychlor, mirex, propanil, trifluralin, 2,4-D and juvenile hormones. We also supported research on the effects of methoxychlor and mirex on embryonic, larval, juvenile, and adult striped mullet, *Mugil cephalus* (Lee et al., 1975).

Research at Gulf Breeze on sensitive stages of estuarine organisms concentrates primarily on larval and postlarval grass shrimp (*P. pugio*), and embryos and fry of the fishes *C. variegatus*, *Fundulus similis*, *F. heteroclitus*, *Leiostomus xanthurus*, *Menidia menidia* and *Morone saxatilis*. Recent papers on this research include those of Hansen et al. (1975), Middaugh et al. (1975), Parrish et al. (1976) and Schimmel et al. (1975). This research focused primarily on effects of toxicants in water on development and survival of early life-stages of these species. On the other hand, investigations (Hansen et al., 1973) of effects of a PCB, Aroclor® 1254,* in eggs of the sheepshead minnow, *C. variegatus*, indicated that concentrations in excess of 5 μg/g of the PCB in eggs were lethal to embryos and fry (Fig. 1). If this PCB has similar effects on other fishes residues exceeding 5 μg/g in eggs would decrease survival of fry.

Life-Cycle Bioassays

Life-cycle bioassays are routinely conducted by freshwater toxicologists, but saltwater toxicologists have developed similar procedures only recently. Such freshwater bioassays can be conducted with bluegills (*Lepomis macrochirus*), fathead minnows (*Pimephales promelas*), brook trout (*Salvelinus fontinalis*), water fleas (*Daphnia magna*), and other fishes and invertebrates (Eaton, 1973).

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*Registered trademark, Monsanto Company, St. Louis, Missouri. Mention of trade names does not constitute endorsement by the Environmental Protection Agency.

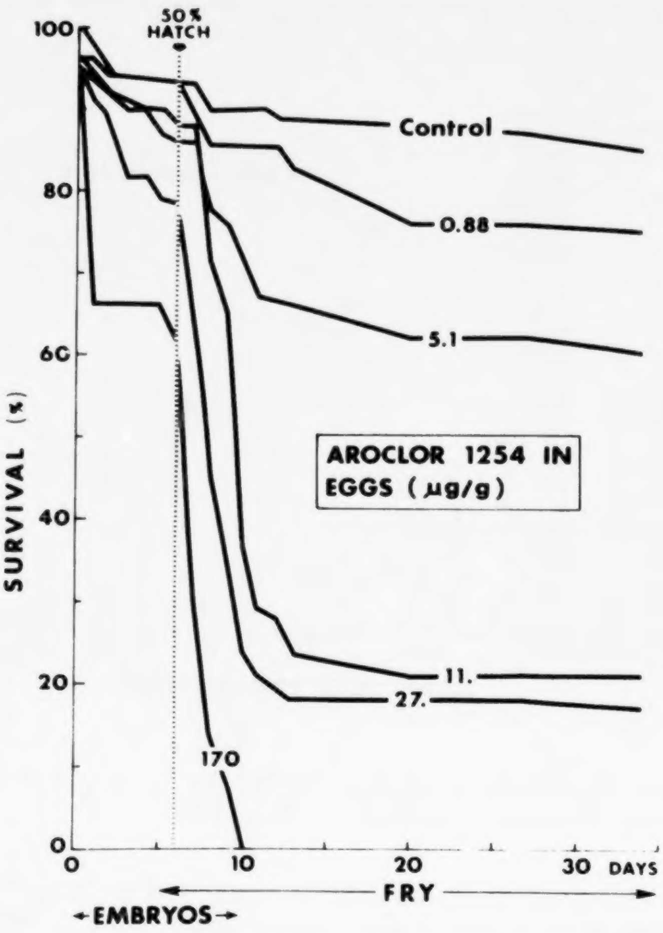

Figure 1. Effect of Aroclor 1254 in eggs of sheepshead minnows on the survival of embryos and fry in laboratory bioassays.

Entire life-cycle bioassays are possible with the estuarine fish, *C. variegatus* (Schimmel and Hansen, 1975). In the laboratory, this oviparous fish develops from an embryo to maturity in about 10 weeks, with about 70% surviving. The fish spawns readily in an aquarium, producing about 8 eggs per day (Fig. 2). The size of the fish apparently has no effect on total egg production but does influence the frequency of spawning and egg fertility (Schimmel and Hansen, 1975). Females begin producing eggs at 27 mm standard length. In one experiment, 19 fish less than 35 mm long produced an average 8.2 eggs per day; 15 fish, 35 mm and longer, averaged 7.8 eggs per day. Smaller fish produced eggs more consistently than larger fish (50% of the days vs 31%) and with greater fertility (94% fertility vs 79%). These and other observations lead to a tentative method for entire life-cycle bioassays using this fish (Hansen and Schimmel, 1975).

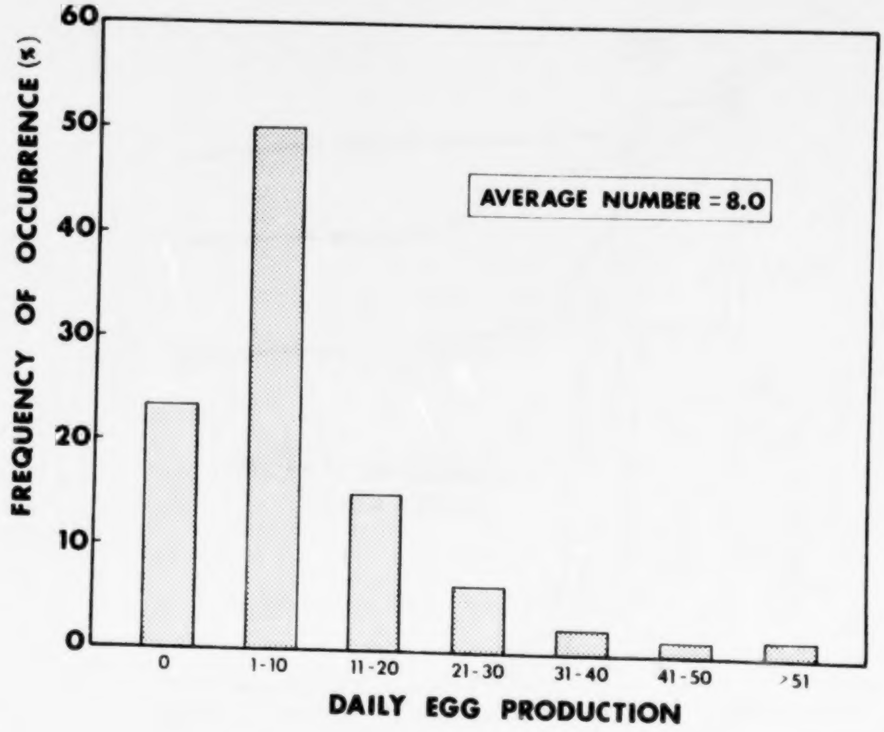

Figure 2. Frequency distribution of egg production by breeding pairs of sheepshead minnows (*Cyprinodon variegatus*).

Recently, sheepshead minnows were exposed to endrin and to heptachlor to determine the effect of these pesticides on reproduction. In one experiment, sheepshead minnows were exposed to 0, 0.025, 0.077, 0.12, 0.31 or 0.77 µg/l of endrin measured in water during a life-cycle bioassay lasting 25 weeks. This bioassay consisted of three parts: (1) The exposure began with embryos and continued through embryonic development, hatching of fry and growth of the fry to adulthood; (2) exposure continued during monitoring of spawning success, including egg production and fertility; and (3) the bioassay ended after embryos and fry obtained from spawning fish were exposed for 28 days. The apparatus used in the experiment was developed by Schimmel et al. (1974) and the methodology was similar to that of Hansen and Schimmel (1975).

Results of this bioassay showed that sheepshead minnows were affected by endrin (Table 2). Embryos in 0.31 and 0.72 µg/l hatched in a shorter period than embryos in water free of endrin. Fry in 0.72 µg/l began to die one day after hatching; more than half were dead by day 12. Survival of juvenile fish was unaffected. Survival of spawning females in 0.31 µg/l was reduced and their eggs were less fertile than those of control females. Survival decreased for fry from eggs spawned by fish that were exposed to 0.31 µg/l of endrin throughout their lives.

TABLE 2. EFFECTS OF ENDRIN ON SHEEPSHEAD MINNOWS (*CYPRINODON VARIEGATUS*) EXPOSED THROUGHOUT THE ENTIRE LIFE-CYCLE. CONCENTRATIONS TESTED WERE: 0, 0.025, 0.077, 0.12, 0.31 AND 0.72 µg/l.

Generation	Life Stage	Effect	Concentrations (µg/l)
F_1	Embryo	Early hatching	0.31, 0.72
	Fry	Death	0.31, 0.72
		Decreased growth	0.31
	Juveniles	No effect	
	Adults	Death of spawning females	0.31
		Decreased fertility of eggs	0.31
F_2	Embryos and fry	Death	0.31

Effects of technical heptachlor on reproduction and development of *C. variegatus* were studied in a similar experiment beginning with juvenile fish, rather than embryos. Concentrations of technical heptachlor (heptachlor and trans-chlordane) measured 0.71, 0.97, 1.9, 2.8 and 5.7 µg/l. During the first four weeks, some juvenile fish died in 2.8 and 5.7 µg/l of technical heptachlor. Thereafter, few exposed fish died until the reproductive portion of the experiment in week 8. Heptachlor also reduced number of spawnings, number of eggs, fertility of the eggs and survival of fry from fertile eggs.

Techniques are being developed for life-cycle bioassays using the grass shrimp (*P. pugio*). Studies have been conducted to determine how light and temperature affect initiation and success of spawning. Larval and post-larval shrimp were used in bioassays to observe effects of certain PCB's on larval development and metamorphosis. *P. pugio* spawns readily, the larvae develop normally and the species is sensitive to toxic chemicals. Therefore, the species may be useful for entire life-cycle bioassays.

C. COMMUNITY BIOASSAYS

Bioassays can be used to predict how communities of estuarine organisms may respond to a toxicant. Bioassays exposing only one species to a chemical may help predict how a community may respond if a number of species from various phyla can be tested under similar conditions. However, predictions made from this type of data are questionable, particularly if little is known about how species interact in the community. Predictions based on data obtained from field studies also may be questioned because of problems related to lack of controls and replication. As an alternative, laboratory tests could study how communities of organisms react when exposed to a chemical.

This approach can be valuable if laboratory communities resemble those in the field and if replicates and concentrations are adequate for statistical analyses.

I have completed two bioassays to determine the effects of Aroclor 1254, and of toxaphene, an organochlorine insecticide, on the development of estuarine communities. The numbers, species and diversity of animals that grew from planktonic larvae in contaminated aquaria were compared with those that grew in identical uncontaminated aquaria. In each bioassay, seawater, with its natural complement of plankton, flowed into 40 sand-filled aquaria; 10 for each of three toxicant concentrations and a control. Planktonic larvae colonized the sand and walls of each aquarium. At the end of the experiments (4 months for the PCB and 3 months for toxaphene) organisms were collected in a 1 mm-mesh sieve, preserved and identified.

Composition of communities in control aquaria differed from communities of estuarine animals that developed from planktonic larvae in salt water that flowed through 10 aquaria contaminated with 1 or 10 µg/l of Aroclor 1254 (Hansen, 1974). Communities in control aquaria and aquaria that received 0.1 µg/l of PCB for four months were dominated (>75%) by arthropods, primarily the amphipod *Corophium volutator* (Fig. 3). Aquaria that received 1 or 10 µg/l of PCB contained fewer arthropods. The dominant species were chordates, primarily the tunicate *Molgula manhattensis*. Over 75% of the animals in aquaria that received 10 µg/l were tunicates. Phyla, species, and individuals (particularly amphipods, bryozoans, crabs, and mollusks) were present in fewer numbers in aquaria receiving the PCB. The abundance of annelids, brachiopods, coelenterates, echinoderms or nemerteans was apparently unaffected (Table 3). The Shannon-Weaver index of species diversity was not altered by Aroclor 1254 in this experiment. Therefore, its usefulness in assessing PCB-induced changes in community structure in the environment may be inappropriate.

TABLE 3. EFFECT OF AROCLOR 1254 ON THE NUMBERS OF PHYLA, SPECIES AND INDIVIDUALS AND ON THE SHANNON-WEAVER INDEX OF SPECIES DIVERSITY IN COMMUNITIES OF ESTUARINE ORGANISMS THAT DEVELOPED IN SAND-FILLED AQUARIA DURING A 4-MONTH BIOASSAY

	Control	Aroclor 1254 µg/l		
		0.1	1	10
Phyla	9	7	7	5*
Species	52	34	43	25*
Individuals	1776	2043	1421	657
Species diversity index	1.82	1.26	2.21	1.70

*Statistically different from controls, $\alpha = 0.05$.

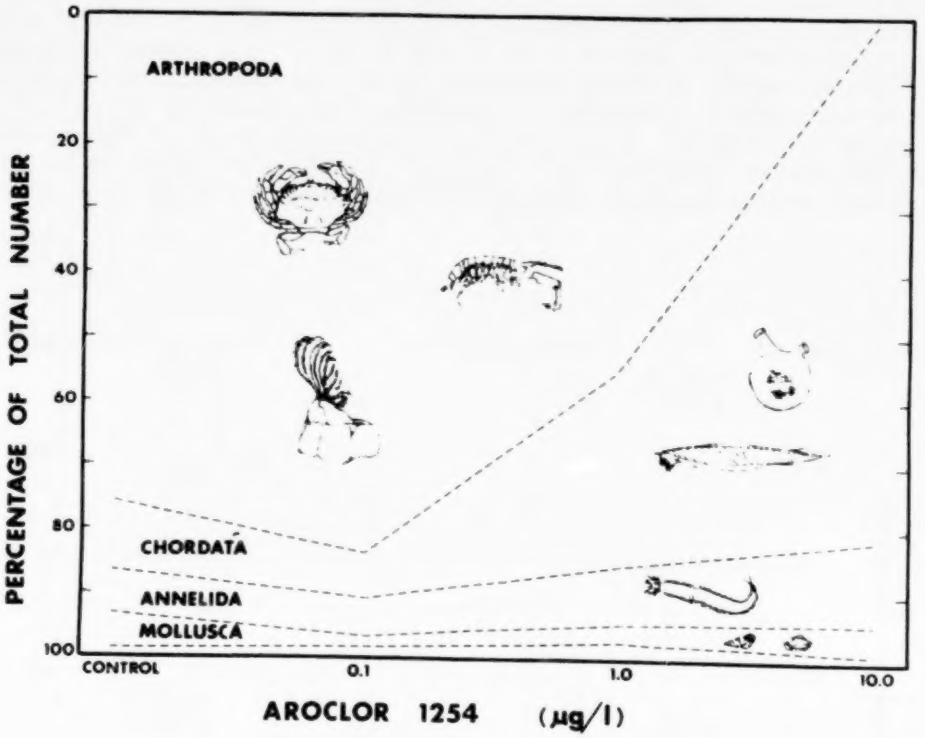

Figure 3. Percentage of organisms from various phyla in communities of estuarine organisms that developed in the presence of Aroclor 1254.

TABLE 4. AVERAGE NUMBER OF ANIMALS IN 10 CONTROL AQUARIA AND 10 AQUARIA THAT RECEIVED 0.1, 1 or 10 µg/l OF TOXAPHENE FOR THREE MONTHS. RANGES IN PARENTHESES.

Phylum	Control	Toxaphene (µg/l)		
		0.1	1	10
Mollusca	124 (65-146)	170 (98-274)	142 (65-237)	373 (245-489)
Annelida	56 (19-97)	62 (33-90)	66 (31-126)	110 (82-182)
Arthropoda	32 (2-257)	155 (1-523)	9 (1-63)	0.4 (0-1)
Coelenterata	3 (0-21)	3 (0-19)	10 (0-44)	--
Other	0.1 (0-1)	0.1 (0-1)	--	--

In a similar experiment using toxaphene, the structure of communities that developed in sand-filled aquaria differed from those in control aquaria. Exposure concentrations were 0, 0.1, 1 and 10 µg/l. The number of mollusks (primarily gastropods) tripled, annelids (primarily capitellids) doubled, and arthropods were almost eliminated in aquaria contaminated by 10 µg/l of toxaphene (Table 4). Similar numbers of pelecypods were found in all aquaria; however, the height (distance from hinge to distal valve edge) of Morton's cockle (*Laevicardium mortoni*) was significantly reduced by 10 µg/l (Fig. 4).

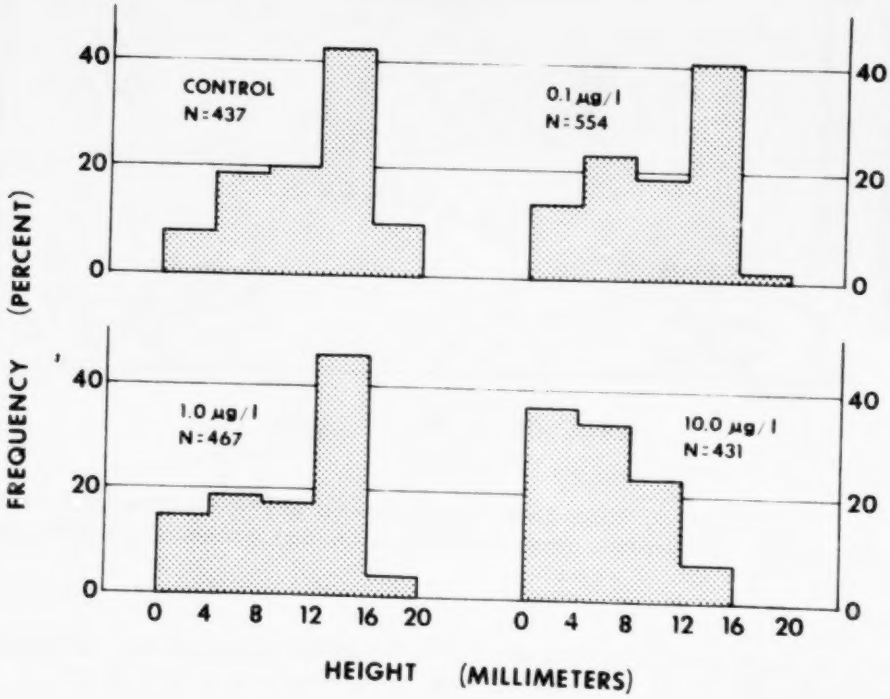

Figure 4. Height (distance from hinge to distal edge of valve) of Morton's cockle collected from a community of estuarine organisms that developed while exposed to toxaphene.

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OIL AND MARINE ORGANISMS

by

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Protecting the coean from pollution is now an urgent international concern. All kinds of wastes originating in man's economic activity sooner or later end up in seas and oceans. This changes seawater chemistry and upsets ecological relationships in the ocean; eventually, this can disrupt the ocean's productivity.

Biologically speaking, the most dangerous and currently the most common pollutant in seas and oceans is hydrocarbons, primarily petroleum and petroleum products. From tanker cargos alone, each year several million tons of petroleum and petroleum products are dumped into the oceans; this is comparable to the quantities of hydrocarbons that oceans produce in photosynthesis. Petroleum prospecting and recovery in the continental shelves is going on at a furious pace, leading to more pollution of the ocean. As oil tanker traffic grows, as bigger tankers are built, the added danger of accidents develops; if accidents happen, tens and hundreds of thousands of tons of petroleum products may end up in the ocean at the same time. Besides the direct damage that these catastrophes inflict, large sums are spent in eliminating the after-effects of tanker spills. The *Torrey Canyon* wreck off the British coast in March 1967 was one such example.

Spilling into the ocean, petroleum can be swept many thousands of miles from spill sites, gradually penetrate into the ocean depths, accumulate in the seabed, and then surface again. So we see that petroleum acts on all groups of marine organisms inhabiting both the surface film and the ocean depths and the seabed.

Oil threatens primarily the early developmental stages of marine organisms. Larvae of numerous hydrobionts perish in ocean water containing petroleum at concentrations of several milligrams per liter. Fish eggs are particularly susceptible to petroleum. When petroleum is present at a concentration of 0.01 mg/liter, the number of nonviable larvae emerging from developing eggs grows by a factor of severalfold.

Petroleum has a damaging effect on marine organisms in short exposures (minutes or hours), killing hydrobionts even if they are in clean seawater after an exposure. Alterations in flora and fauna caused by pollution are well known. These rearrangements in aquatic communities are observed both because of pollutants continuously spilled into the seawater in small amounts and in massive spills. In the first case, the changes are gradual and become noticeable only after many years. This process can be slowed down by fluctuations in the rate of pollution.

Typical of accidental oil spills is the disruption, and even the death, of entire biocenoses in a short time, for example, in the wreck of tankers, the rupture of underwater oil pipelines, and so on.

Noteworthy is the fact that petroleum's effects on the rearrangement of marine communities often stretch beyond the immediate influence of the pollutant; later, alterations in flora and fauna occur even when no hydrocarbons are directly involved. Diesel fuel spills from the Japanese tanker *Timpako Maru* along the California coast significantly cut into the populations of sea urchins and mollusks feeding on algae. The profuse growth of the giant alga Macrocystis ensued. When the sea urchin population was restored, the area occupied by the Macrocystis--the primary food of the sea urchin--was restored to its original extent.

A system of observation and monitoring of oil contamination that relies on marine organisms, that is, biological monitoring, can be important in this respect.

By relying on a series of hydrobionts beloning to different systematic groups, and biocenoses and communities of marine organisms, observations of oil pollution can be made over practically all the world's oceans. And biological methods of global observation can and must be combined with several biological tests available for individual bodies of water; this makes it possible to track the dynamics of pollution and various transformations of an organic mixture that is as complex as petroleum, as well as the principal petroleum products. Also, this system of biological monitoring permits the evaluation of the role of marine hydrobionts in processes of the natural transformation of hydrocarbons, that is, self-cleaning.

Self-cleaning is a complex process: constituents of pollution break up and participate in the overall turnover of matter and the transfer of energy in the ocean. This process, originating in nature, existed long before man began polluting his environment and even before man appeared on the globe. However, we must emphasize that the ocean's ability to transform hydrocarbons and other kinds of pollution is not boundless. Today numerous bodies of water have already lost their ability to self-clean. In some gulfs and bays, petroleum has accumulated in large quantities in the seabed, transforming them into virtual biological deserts.

Let us look at several examples of biological monitoring of petroleum pollution. Systematic observations we began (Mironov, 1965, 1970, 1971, 1975) since 1967 in studying the population, distribution, species composition, and biochemistry of microorganisms capable of assimilating petroleum hydrocarbons as the sole source of carbon and energy revealed the following:

1. A direct relationship was noted between the population of petroleum-oxidizing microorganisms and the rate of petroleum pollution of

seawater (Table 1). The largest number of cultures was found in the oil pollution regions; and the population of bacteria feeding on petroleum was 10^6-10^7 bacteria per liter of seawater.

2. In addition to the emphasis on population, there is species diversity of microorganisms in the sites of continuous petroleum pollution. The latter, in all likelihood, can be attributed to the great complexity of the chemical composition of petroleum; its constituents can be consumed only by certain microorganism species.

3. The relationship between population level and species diversity of microorganisms on the one hand, and the rate of oil pollution, on the other, enables us to examine petroleum-oxidizing microorganisms as indicators of oil pollution.

The studies, covering all the main regions of the world ocean, allowed us to place on the second session in the international program of the Joint Study of the Mediterranean Sea (Dubrovniki, Yugoslavia, 1975), the draft "Study of the Distribution and Population of Hydrocarbon-Oxidizing Microorganisms in the Aquatic Environment."

TABLE 1. INDICATORS OF THE DISTRIBUTION OF MICROORGANISMS FEEDING ON PETROLEUM IN THE MEDITERRANEAN SEA

Region of investigation	Number of stations	Number of stations at which growth of microorganisms feeding on petroleum is observed	Number of cultures isolated
Northern region	27	18	26
Southern region	27	10	13
Total	54	28	39

PROGRAM OF RESEARCH ON "STUDY OF THE DISTRIBUTION AND POPULATION OF HYDROCARBON-OXIDIZING MICROORGANISMS IN THE AQUATIC ENVIRONMENT"

Hydrocarbon-oxidizing microorganisms are sensitive indicators of hydrocarbon pollution and have the decisive role in the biodegradation of hydrocarbons in the ocean.

Joint and systematic pursuit of the investigation broadens our knowledge of how far pollution extends in the Mediterranean Sea and allows us to judge the potentiality of the aquatic environment for self-cleaning.

Experience with similar investigations by the Soviet Union in the Black Sea and the Mediterranean Sea and some regions of the world ocean shows that:

1. Carrying out these investigations does not require large material outlays (costs of the laboratory processing of material, including the purchase of equipment, are about 5 million dollars for processing 100 samples and about 1000 rubles for each subsequent 100 samples); the processing can be carried out by all countries (or by most countries) that are participating in the Joint Study of the Mediterranean Sea program (JSMS).

2. This program can be carried out both in full volume and by stages, depending on the equipment available, with unified methods.

3. The present investigation, which should preferably start in 1975, must later be converted into continual systematic observations, to grow in volume year by year.

4. Carrying out these observations in one's national coastal waters can favorably affect the progress of similar studies on an international scale.

STAGE ONE: MINIMUM PROGRAM

1. Determination of the population (by the method of maximum dilutions) of hydrocarbon-oxidizing microorganisms in mineral media with a single source of carbon and energy (crude petroleum and simple phenol). Other hydrocarbons can be used.

   a. Only in the surface layer of the sea, 0-1 m

   b. Surface layer of the sea plus standard levels

   c. Surface layer of the sea plus standard levels plus seabed.

2. The test body of water can include, depending on the opportunities open to the investigator:

   a. Periodic profiles within his country's territorial waters

   b. Observations at permanent coastal stations

   c. Expeditionary studies in regions of the Mediterranean Sea (ships in transit can be used)

   d. Synchronous studies in bodies of water, and so on.

3. The periodicity of observations can fluctuate from daily to weekly, monthly, and so on.

Present investigations can pass at once into the second stage of the program, providing for the isolation from seawater (seabed) of the hydrocarbon-oxidizing microorganisms and the determination of their hydrocarbon-oxidizing activity. This makes it possible to conduct approximate calculations of the self-cleaning activity of the oceans and to predict the extent of pollution of the aquatic environment.

The program was approved by the countries participating in this conference, and Bulgaria, Greece, Spain, and Romania declared their desire for early affiliation with it. Since this program was executed two months after it was presented at the JSMS session, an expedition was undertaken to the Mediterranean Sea aboard the scientific research vessel *Akademik Kovalevskiy*. Carrying out these studies at present is of high interest, both because the Suez Canal was recently opened and because oil shipments across the Mediterranean began.

Besides the microbiological monitoring system in coastal waters, there is much interest in observing the changes in biocenoses and individual groups of marine hydrobionts under the effect of pollution. Here we must add the reservation that in coastal bodies of water changes in the structure of the marine biota in general are caused not by some single pollutant, for example, petroleum, but by a combination action of a number of pollutants. In this respect, to carry out monitoring of petroleum pollution, it is necessary to conduct observation of the level of accumulation of petroleum hydrocarbons in aquatic organisms. Naturally, a convenient object here is the filter feeders. For example, the bivalve mollusk mussel *Mytilus* can prove promising in this respect; they are capable of carrying off quite high concentrations of petroleum in seawater. However, this system of observation at the present stage cannot be recommended for broad global use (as microbiological monitoring) owing to certain technical difficulties. These analyses are within the capability of well-equipped laboratories that have appropriate specialists on their staffs.

In discussing observations of a particular pollutant, we must note that complete information on the pollutant can be obtained from an integrated study of the environment. As an example, we can point to multi-annual studies we conducted (Mironov et al., 1975) in the Black Sea. The study of the population, species composition, and biochemical features of petroleum-oxidizing microorganisms in seawater and in the seabed, and physicochemical properties and processes of the transformation of the organic matter in seabed deposits helped to produce not only material on the present status of the body of water, but also helped to evaluate the possibility of self-cleaning in a vast region of the Black Sea. From our calculations, the self-cleaning potentiality of the coastal body of water with respect to petroleum (to depths of 100 m) embracing the region of the Soviet coast of the Black Sea is about 200 tons of petroleum a year.

Thus, in a system of biological monitoring of hydrocarbon pollution of the ocean environment, one can suggest a method of determining the hydrocarbon-oxidizing microorganisms that does not require large amounts of money and can be effected on a global scale and in individual marine bodies of water.

In coastal bodies of water, a method of studying the dynamics of hydrocarbon content in marine hydrobionts can prove highly promising. This method is applicable also in open regions of the sea in observations of accidental oil spills.

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IMPACT OF METALS ON THE MARINE ENVIRONMENT

by

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ABSTRACT

Scientists have been unable to present evidence that heavy metals are adversely affecting the inhabitants of the marine environment. Metal-organism interactions are extremely complex and thus difficult to understand. The results of small-scale laboratory experiments have not been extrapolated to the marine environment because the levels used in the laboratory generally have been of higher magnitude than those occurring in nature. The findings of elevated concentrations of heavy metals in marine organisms has led to the detection of hot spots. However, it has been impossible to discern whether the organisms were adversely affected by these metals.

The need for new methodology for evaluating the impact of heavy metals has led to large-scale ecosystem experiments in which delicate marine organisms were maintained under near natural conditions. In initial experiments with plankton, adverse metal effects were observed at concentrations approaching ambient levels--e.g., 5 ppb Cu, 0.25 ppb Hg. These results, in turn, reemphasized the need to determine metal concentrations accurately in seawater. Fortunately, the state-of-the-art for such measurements has constantly improved, primarily because of sensitive new analytical techniques, the use of clean collection, and analytical procedures. Thus, the continued development and usage of large-scale ecosystem experiments in conjunction with accurate measurements of heavy metal levels in seawater should enable scientists to assess the impact of heavy metals on the world's oceans.

INTRODUCTION

The toxicity of heavy metals has been the subject of a vast amount of research, primarily because of their threat to human health. For example, several disastrous cases of mass poisonings by mercury compounds have occurred in recent years. Between 1953 and 1960, 121 cases of Hg poisoning were recorded in Minamata, Japan; 46 people died. In Iraq mass Hg-poisoning epidemics occurred in 1956 (100 cases, 14 deaths); in 1960 (1,000 cases), and in 1972 (6,530 cases, 459 deaths) (Eyl, 1971; Bakir et al., 1973).

Several other heavy metals are also toxic enough to represent human health hazards. Cadmium-induced decalcification of the skeleton (Itai Itai Disease) claimed 56 lives in Japan (Nilsson, 1970). Human lead poisoning has been known since antiquity (Patterson, 1965).

In addition to the threat to mankind, agricultural scientists have been concerned as to how the toxicity of certain trace elements affects valuable domestic animals. This is best exemplified by selenium, an element whose toxic effects were apparently first described in 1295 by Marco Polo during his travels through China (Trelease and Beath, 1949). This element is concentrated by certain plant species, and herbivores grazing on these plants exhibit symptoms commonly called "alkali disease" and "blind staggers." These conditions can lead to mass mortalities; in 1906 and 1908, 15,000 sheep died of selenium poisoning in the state of Wyoming (Trelease and Beath, 1949).

Mass mortalities caused by heavy metals have also been observed in the marine environment. Approximately 2,000 abalone (*Haliotis rufescens, H. cracherodii*) died from copper poisoning when a power plant cooling system was tested (Martin et al., 1975b). The copper leached out of copper-nickel tubing into seawater that had stood in the system for several weeks. When the water was released, it contained approximately 2,000 ppb Cu, a concentration that resulted in the deaths of almost all abalone in the immediate discharge area.

However, in comparison to the terrestrial environment, such instances rarely have been reported in the marine environment. Neverthless, research efforts on metals in the sea increased in recent years because of a series of interrelated events. The well-publicized Minamata Bay disaster was caused by the consumption of marine food items contaminated with large amounts of methyl mercury. A few years later, analytical capabilities for tracing elements were advanced by the development of a very sensitive technique for detecting Hg (Hatch and Ott, 1968). This breakthrough led to the discovery that important marine food items, such as tuna and swordfish, had relatively large quantities of Hg. Consequently, man became increasingly concerned not only with the quality of food items from the sea, but also with the quality of the marine ecosystem itself. The combination of these events resulted in increased heavy-metals research in the marine environment.

PAST RESEARCH EFFORTS

Previous research efforts can be divided into two areas: (1) laboratory experiments and (2) measurements of heavy metals in organisms inhabiting various parts of the marine ecosystem. A comprehensive review of the laboratory research on the effects of heavy metals is not feasible in a paper of this length. Further, it is unwarranted because excellent reviews on this subject (e.g., Bryan, 1971) and comprehensive bibliographies (i.e., Eisler, 1973, 1975) have been published. However, it is generally apparent that laboratory experiments have been extremely valuable in demonstrating that heavy metal toxicity in the marine environment is a very complex phenomenon. For example, Bryan (1971, Table 4) lists as factors influencing the toxicity of heavy metals: the form of the metal in water (soluble vs. particulate); the presence of other metals or poisons (antagonistic, additive and synergistic effects); the physiology of the organism (salinity, temperature, dissolved oxygen, pH, etc.); and the condition of the organism (stage in life history, its size, its activity, acclimatization). It is not surprising, therefore, that laboratory workers often have been compelled to add relatively large quantities of toxic elements to observe and determine various effects in the laboratory. Nevertheless, this practice drew general criticism that concentrations necessary for producing measurable effects are of a higher magnitude than those encountered in all but the most heavily polluted environments. The failure to detect effects at low levels has prompted some scientists to argue that metals are not a problem in the marine environment. Others contend that, because of the complexities, the effects of metals in the marine environment are not adequately understood. The latter position was considered especially applicable for delicate organisms that cannot survive under ordinary laboratory conditions. Thus, it became apparent that new approaches were needed to assess the full impact of metals in the oceans (see below).

In addition to the experimental area, considerable research also has been conducted to determine the levels of various toxic elements in marine organisms. These studies led to the detection of hot spots--areas in which environmental levels are undoubtedly high. They were also useful in determining the occurrence of food chain amplification processes that might ultimately threaten man as well as other top carnivores. Although these data reveal nothing about whether the animal assimilating concentrations was adversely affected by the element, it appears that most scientists erroneously assume that the higher the levels in an organism, the more likely the chances of adverse effects. Although this assumption is logical, there is growing evidence that it is a fallacy. For example, Friberg et al. (1974, p. 107) state: "When a person suffers severe renal damage through the toxic action of cadmium, his kidney concentration of cadmium will decrease considerably. Thus he will have lower levels in his kidneys than a person with only slight renal disturbances." In discussing their experiments with a marine gastropod, Betzer and Yevich (1975, p. 24) note: "In *Busycon*, experiments using 64-Cu show that under normal concentrations, copper is taken up and transferred to the internal tissues; at toxic concentrations, where only the gills (and osphradium) show tissue damage, the rate of transfer into the internal tissues, particularly the digestive gland, is sharply decreased." Betzer and Yevich also refer to Yager and Harry (1964), who found the livers of distressed fresh water snails, *Taphius glabratus*, had lower copper concentrations than those of normal snails. These few examples suggest that high concentrations of heavy metals in certain organs may indicate that the organisms are in good health and that their detoxification mechanisms are functioning properly. Obviously, a technique involving more than high concentrations is needed to detect metal damage to the environment.

Thus, while using both experimental and environmental approaches, scientists have been hard pressed to illustrate that heavy metals are adversely affecting the marine ecosystem.

PRESENT RESEARCH

As mentioned above, small-scale laboratory experiments were valuable in demonstrating the complex nature of toxic element-organism interrelationships in the marine environment. They also indicated the need for large-scale total ecosystem experiments, in which delicate organisms could be maintained and studied when subjected to realistic amounts of various pollutants. Planktonic organisms were likely choices for such experiments since the phytoplankton are the most important organisms in the marine environment. They are extremely sensitive and thus are likely to be affected by microchanges in their chemical environment.

For these reasons, the controlled ecosystem pollution experiment (CEPEX) program was undertaken under the auspices of the National Science Foundation's International Decade of Ocean Exploration Program (NSF-IDOE). Initial experiments have been performed in the Saanich Inlet off Vancouver Island, British Columbia (48°39'N, 123°28'W). Approximately 70 tons of natural water are isolated in control and experimental polyethylene bags (diameter 2.5 m; length 15 m). The bags are cylindrical in shape except for the lower one-fifth which is conical and tapers to a removable sediment trap at the base. Initially, bagged plankton populations were compared with those living in the waters surrounding the bags. The results suggest that the inside and outside populations and their environments were essentially the same (Takahashi et al., 1975). Thus, populations under pollutant stress situations can be compared meaningfully with control populations.

One of the first pollutants to be tested was copper. The choice was logical since certain aquatic plant species already had responded remarkably to small amounts of this element. For example, inhibition of *Chlorella* growth and photosynthesis was observed at concentrations of one ppb Cu; a reduction in photosynthesis was also observed when fresh water diatom *Nitzschia palea* was subjected to this level. This research was performed by Steemann Nielsen and his co-workers Kamp-Nielsen and Wium-Andersen. In addition to the findings above, these authors also have discussed the importance of copper in upwelling systems and have noted its possible effects on primary productivity (Steemann Nielsen et al., 1969; Steemann Nielsen and Kamp-Nielsen, 1970, Steemann Nielsen and Wium-Andersen, 1970, 1971, 1972).

The initial CEPEX copper experiemtns demonstrated the value of the ecosystem approach since several components of the ecosystem could be observed simultaneously when 10 and 50 ppb Cu were added to experimental bags. The following events occurred: The number and activity of bacterial heterotrophs increased markedly, apparently in response to increases in carbon from other copper-stressed components of the ecosystem (Vaccaro et al., 1975); phytoplankton crops and photosynthesis were inhibited by copper; and the excretion of ^{14}C-labeled organic matter increased (Thomas et al., 1975). The species composition of the phytoplankton was also affected: Populations of the centrate diatom *Chaetoceros* sp. declined and were replaced by microflagellates and Cu-insensitive diatom species such as *Nitzschia delicatissima* and *Navicula distans* (Thomas and Seibert, 1975). Phytoplankton nitrate uptake and the synthesis of nitrate reductase were inhibited. Cell disruption and loss of accumulated ammonium was observed in *Noctiluca* sp. (Harrison et al., 1975). The copper caused decreases in the zooplankton standing crop. Larvaceans appeared to be the most sensitive of the organisms present during the experiments (Gibson et al., 1975). Other interesting effects also were noted in the zooplankton population. For example, copepod fecal pellet production dropped markedly as copper concentrations were increased (Reeve, 1975). An interesting interaction between zooplankton predators and their prey also was noted. Ctenophores and coelenterates declined in abundance in the experimental bags, while those in the control bags increased. Consequently the abundance of prey species declined markedly. These interactions made it difficult to evaluate the effect of Cu on the zooplankton (Gibson et al., 1975). Decreases in microplankton were also observed. Copepod nauplii were affected at concentrations as low as 5 ppb (Beers, 1975).

Although not yet reported, further CEPEX experiments have been conducted with lower concentrations of Cu (5 ppb). Similar results were obtained. Many identical changes also were observed when concentrations of 0.25 and 1.0 ppb Hg were used (CEPEX Newsletter, 1975). The CEPEX experiments thus demonstrate the value of the large ecosystem approach revealing that subtle, often sublethal effects occur when metals are added to an ecosystem. Clearly more large-scale ecosystem experiments are needed; and it is encouraging to note that the construction of another project is now underway at the Environmental Research Laboratory, Narragansett, Rhode Island, under the auspices of the Environmental Protection Agency.

THE NEED FOR TRACE METAL DATA IN SEAWATER

The effects of heavy metals in the marine environment will never be adequately understood until accurate measurements of heavy metals in seawater are obtained: For example, if laboratory experiments indicate an effect at a concentration of one ppb, it then becomes necessary to define areas where this concentration is exceeded. These data also are needed to determine transport pathways, residence times, mixing rates, etc. In recent years, the state-of-the-art in water chemistry has constantly improved. As a result, metal concentrations in seawater are now believed to be much lower than originally estimated. For example, copper values for open-ocean seawater formerly were believed to be approximately 2-3 μg/l. However, in a recent paper, Boyle and Edmond (1975) observed a range of 0.06-0.21 μg Cu/l of Antarctic surface water. Further, they reported a strong correlation between Cu and nitrate. If Cu is correlated with nitrate throughout the world's oceans, open-ocean surface waters depleted by nitrate should have less than 10 ng Cu/l. The maximum Cu concentration in deep ocean water should be 300 ng/l. Martin et al. (1976) observed a similar correlation between Cd and nitrate and phosphate. Their Cd value for open-ocean surface water was 0.005 μg/l, a value 20 times lower than the generally accepted level of 0.1 ppb.

These low values were obtained by using clean sampling and laboratory techniques. Perhaps sampling is the greatest source of contamination. Large quantities of trace metals are constantly sloughed off all oceanographic research vessels; the hydrowire attached to samplers is often filthy, and even the best commercial samplers contain metal parts or are constructed of materials known for high metal content. These samples are difficult to clean properly at sea, if indeed they are cleaned at all between stations. Because of contamination problems, scientists have devised new sampling techniques. Boyle and Edmond collected their samples by lowering a carefully cleaned polyethylene bottle from the bow of a research vessel. The bottle was suspended on a polypropylene line. Martin et al. (1976) collected samples by rowing a raft away from the research vessel and then hand-holding a carefully cleaned polyethylene bottle beneath the sea surface. They also employed a specially constructed metal-free pump system to collect water samples as deep as 95 m. Obviously, these methods are not suited for collecting deep-water samples. A clean reliable deep-water sampler has yet to be built.

Water samples also require special handling in the laboratory. C. C. Patterson of the California Institute of Technology has led attempts to persuade scientists to follow clean collection and laboratory procedures for trace elements, especially lead. He has long maintained that estimates of Pb in ocean water were exaggerated by several orders of magnitude because of contamination during the collection, transport, and analyses of these samples. Patterson convinced many scientists of the need for special procedures during a lead-in-seawater-intercalibration workshop (see Meeting Report, 1974). Water collected from the surface of the heavily polluted Southern California Bight contained only 0.014 μg Pb/kg. Other laboratories participating in this workshop reported concentrations higher than this value. The excess lead was thought to be introduced during analytical processes in which unpurified reagents were used in unclean facilities. Patterson has proven his point. Marine chemists should adopt his procedures whenever feasible unless they can demonstrate that such stringent requirements may be safely omitted in certain situations for certain elements. In any event, scientists involved in trace-element seawater chemistry are urged to read Patterson and Settle's (1976) article before beginning or continuing this kind of research.

In conjunction with seawater data, anthropogenic and natural input rates must be obtained for heavy metals in the world's oceans. These data are presently scarce, but steps are being taken in this direction. For example, Duce and his co-workers in the NSF-IDOE Pollutant Transport program are providing a great deal of information about the fluxes of metals to the world's oceans via atmospheric fallout (e.g., Duce et al., 1975; Hoffman et al., 1974; Wallace and Duce, 1975). Estimates of total input are available for at least one geographical area. Young and his co-workers provided input rates for several heavy metals via wastewater discharge, surface runoff, vessel coating, ocean dumping, rainfall, and advective transport into the Southern California Bight (SCCWRP, 1973, 1974, 1975). Such vital studies are needed in other world population centers that border the marine environment.

WHO KILLED COCK ROBIN?

Scientists involved in environmental pollution research are often required to perform detective work. For example, mortalities or deleterious effects are observed for a group of organisms, and the question arises: Was an environmental pollutant responsible, and if so, which one? Answers to such questions are very elusive because complexities are almost always encountered. E. D. Goldberg of the Scripps Institution of Oceanography has termed this phenomenon the "who-killed-cock-robin syndrome."

This situation is exemplified by studies of premature pupping in the California sea lions (*Zalophus californianus*). Large numbers of premature pups have been counted on the sea lion rookeries since 1968 (see Gilmartin et al., 1975; Odell, 1970). Research aimed at determining the causes for this development have revealed: (1) The mothers of premature pups are usually only 6-8 years old, while the mothers of full-term pups are at least 10 years old (Gilmartin et al., 1975); (2) many of the abnormal mothers are infected with Leptospirosis, a virus known to cause abortions (Vedros et al., 1971; Gilmartin et al., 1975); (3) the abnormal mothers have significantly higher amounts of polychlorinated biphenyls (PCB's) and DDT compounds (DeLong et al., 1973; Gilmartin et al., 1975); and (4) the normal mothers have significantly higher amounts of mercury, selenium, and bromine (Martin et al., 1975a). The latter findings were of interest because each normal mother had equimolar amounts of Se and Hg in their livers; and, in addition, excess or equimolar amounts of Br were found in conjunction with these elements. In contrast, the mothers of premature pups had equimolar amounts of Se and Hg; however, their Br levels were severely depressed. Perhaps these findings indicate that Br also is involved in the Hg-Se detoxification mechanisms (see Parizek et al., 1971) although for some reason it was not functioning in the abnormal mothers. Whether it was responsible for the premature pupping is unknown. However, these results suggest that absolute amounts of elements are not as important as the relationship of elements.

In addition to demonstrating the complexities involved with environmental detective work, the four factors mentioned above also point to the desirability of simultaneous measurement of different pollutant classes as well as natural factors within the same samples. Erroneous conclusions can be reached when only one pollutant is measured. As the Se-Hg interaction indicates, this risk is especially true for one heavy metal.

From the limited overview presented above, we can conclude that the impact of heavy metals in the marine environment is exceedingly difficult to understand. Nevertheless, recent developments in large-scale experimental methods and in analytical capabilities provide us with powerful tools that will lead to the desired understanding. In the meantime, no one can justifiably state that metals are or are not a problem in the world's oceans.

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APPENDIX

MICROCOSMS AS BIOLOGICAL INDICATORS OF POLLUTION

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ABSTRACT

Microcosms are one method of investigating specific origins, flows, fates, and/or effects of materials in the environment. The EPA Gulf Breeze Laboratory conducts and supports research to develop microcosms of many types and complexities. These microcosms are intended to be simple, easy to apply, and are designed to investigate specific processes or categories of processes in the estuarine environment. The objective of this research is to develop a number of methods to investigate pollutant fate and effects in estuaries. The results of the individual tests are combined to form a description of the entire system. The ecosystem compartments under investigation include direct accumulation from water and food by organisms at all trophic levels, bioaccumulation through food chains, direct effects of pollutants on organisms, i.e., mortality, reproduction and behavior and indirect effects of sublethal levels of pollutants such as changes in predator-prey relationships. Microbial processes at both air-water and sediment-water interfaces are investigated as well as physical and chemical transformations.

Specific tests under development include:

1. Predator/Prey Effects Test in which prey selection and the ability of the prey to avoid predation is affected by the pollutant.

2. Lugworm Benthic System in which pollutants are accumulated by these important benthic organisms and mobilized into the soil through their activity.

3. Model Salt Marsh Ecosystems in which pollutant effects are determined on microcosms which simulate seasonal and tidal conditions.

4. Microbial microcosms in which the degradation of pollutants by naturally occurring microbial populations is investigated as well as the effect of the pollutant on population diversity and composition.

5. A "Slow-Flow" System in which the fate of pollutants is determined in a sealed, continuous air and water flow, aquarium containing a representative estuarine community.

6. A Gradient Controlled System in which the avoidance and/or preference of estuarine organisms to pollutant gradients is determined.

7. A Food Chain System in which the bioaccumulation of pollutants through typical estuarine food chains is determined.

REPORT OF THE JOINT AMERICAN-SOVIET EXPEDITION ON THE SCIENTIFIC RESEARCH VESSEL "MOSCOW UNIVERSITY" FOR THE FIRST STAGE OF INTERCALIBRATION OF METHODS FOR THE HYDROBIOLOGICAL ANALYSIS OF AQUATIC ECOSYSTEMS

by

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INTRODUCTION

In accordance with the American-Soviet Environmental Agreement within the framework of Project VI-2.1, "Effect of Pollutants on Marine Organisms," a protocol was signed expressing the desire to conduct an intercalibration of hydrobiological methods for the analysis of aquatic ecosystems. The first stage of the intercalibration was a comparison of American and Soviet methods for measuring phytoplankton productivity which was conducted aboard the Research Vessel *Moscow University* in the western Atlantic Ocean and eastern Gulf of Mexico during summer, 1975.

Previous international intercalibration of the carbon-14 phytoplankton primary productivity method devised by Steemann-Nielsen (1952) had revealed differences in productivity data obtained in the Indian Ocean by American, Soviet, Japanese, and Australian investigators (Doty et al., 1965). The differences were related to variations in choice of sampling depth, in-situ incubation apparatus, standardization of carbon-14 stock solutions, and in the determination of Geiger counter efficiencies. Since we were aware of the results of the Indian Ocean phytoplankton productivity intercalibration, and since new methods have been developed for processing carbon-14 labeled phytoplankton material since 1965, attention was focused in the intercalibration reported here on effects of differences in in-situ incubation gear, filters and filtration equipment, and liquid scintillation processing of samples. Liquid scintillation methods have been developed to the point where they are in wide use for estimation of phytoplankton productivity (Wolfe and Schleske, 1967). Liquid scintillation counting of carbon-14 labeled phytoplankton can be accurately accomplished if absorption by phytoplankton is assessed and corrected for (Pugh, 1970).

SAMPLING, SAMPLE PREPARATION, AND INCUBATION OF SAMPLES

Water samples were collected with well-aged 5-liter Niskin water bottles from 5 arbitrarily chosen depths at each of 10 stations in the western Atlantic Ocean, Florida Straits, and eastern Gulf of Mexico. Only light bottles were used for this intercalibration since Morris et al. (1971) suggested there is little justification for dark bottle corrections to light bottle carbon-14 fixation in the open ocean. The American method used three 180 ml glass bottles which were filled with water from a Niskin bottle for each sample depth. Two milliliters of solution containing 1 x 10^7 disintegrations per minute (DPM) of carbon-14 as $NaHCO_3$ were then added to each bottle with a Cornwall automatic syringe. The bottles were capped, shaken, and placed within a clear plastic tube attached vertically to the hydrographic line with a clamp. The samples were lowered into the sea and incubated for periods of about 3 hours at the depths where they were collected. After the incubation period, the samples were returned to the deck of the ship where one ml of a 2% solution of $HgCl_2$ was added to each bottle to kill the phytoplankton. Care was taken to avoid exposure of water samples to direct sunlight while the bottles were filled and before phytoplankton were killed.

Soviet productivity bottles were about 125 ml in volume and were filled alternately and in triplicate from the same Niskin bottle from which corresponding American samples were obtained. A carbon-14 stock solution supplied by the Americans was used by both the Soviets and Americans in order to remove carbon-14 standardization problems from consideration in the intercalibration. Soviet bottles were suspended on a horizontal platform made of Plexiglas which was attached to the hydrographic wire at each sample depth. Soviet and American suspension devices were placed in the top position on the hydrographic line in alternate experimental incubations. Soviet samples were kept in the shade after incubation until formalin was added to kill the phytoplankton.

The titration alkalinity method given in Strickland and Parsons (1972) was performed for samples obtained from each Niskin bottle by American and Soviet scientists using American equipment.

SAMPLE PROCESSING

Poisoned American productivity samples were filtered through 24 mm diameter Whatman GF/C glass fiber scintillation grade filters under low vacuum. The filters were exposed to HCl fumes for 10 seconds before being frozen for transport to shore. On shore, samples were thawed and placed in liquid scintillation vials to which 10 ml of Aquasol® (New England Nuclear) were added. Radiocarbon activity was measured with a Picker liquid scintillation spectrometer using the channels ratio method of standardization. A channels ratio curve was prepared using commercial quenched toluene carbon-14 standards and also using the phytoplankton biomass method of Pugh (1970). The Pugh curve was prepared using phytoplankton from a series of different volumes of water collected in the Atlantic Ocean. Quantities of phytoplankton were so low that little quenching was observed. The carbon-14 stock solution was standardized by adding aliquots of a 1:1000 dilution of the 2-ml radiocarbon "spike" to Aquasol® containing one ml of phenethylamine as given by Iverson et al. (1976).

The Soviets used "Synpor" (Czechoslovakia) membrane filters with an effective pore size of 0.3 microns. After filtration, samples were held in HCl fumes for several minutes before freezing. Filters were kept frozen before analysis in Moscow where filters were thawed, desiccated, and placed in liquid scintillation vials together with 4 ml of scintillation solution which consisted of 4 g PPO and 0.1 g POPOP in 1.0 liter of toluene. Radioactivity of the samples was measured with a Nuclear Chicago Mark II liquid scintillation spectrometer (USA) equipped with an Algotronic computer (Diehl, Germany). The method of Pugh (1970) was used to prepare a standard channels ratio curve.

Since phytoplankton on filters constitutes a heterogeneous system, the filters were removed from the scintillation solution and dried before solubilization to prepare a homogeneous system. The scintillation solution was recounted to measure radioactivity which had eluted from each filter. The dried filters were placed in clean scintillation vials with 0.15 ml of distilled water. After allowing the filters to soak for a short time period, 1.0 ml of NCS solubilizer (Amersham, Searle, USA) was added to each vial. Filters were allowed to remain in the NCS solution for 30 minutes after which 0.03 ml of glacial acetic acid was added to each sample to lower chemiluminescence. The samples were cooled, 15 ml of scintillation solution was added to each vial, and the vial contents were carefully mixed. The samples were held in the dark at 4°C for 12 hours before measurement of radioactivity of the homogeneous solution. For standardization, a quench curve for a solution with a known specific activity was prepared by adding carbon-14 ethanol to filters which contained labeled phytoplankton. The filters were solubilized by the method given above. The activity of the carbon-14 ethanol solution was determined with a standard quench curve obtained with carbon-14 quenched standards (Amersham, England).

RESULTS AND DISCUSSION

There did not appear to be consistent differences in carbon-14 activity for samples processed with American and Soviet methods except for Station 9 where Synpor filters were used by both sides (Table 1). American activity was consistently lower than Soviet activity at all depths for Station 9 suggesting that it is necessary to solubilize the Synpor membrane filters in order to minimize radioisotope absorption problems. Anomalously high activity values (for example, Station 3, Soviet, and Station 7, American) were usually characterized by high standard deviations which reflected the effects of high activity values for one or two of the three replicate samples. These high activity values are unexplained but may be the result of uneven distribution of phytoplankton between replicates or the result of problems in correcting for chemiluminescence. The mean coefficient of variation for American carbon-14 activity values was 21 (range 2 to 123) while the mean coefficient of variation for Soviet carbon-14 activity values was 29 (range 0 to 145).

TABLE 1. CARBON-14 ACTIVITY AND PHYTOPLANKTON PRODUCTIVITY VALUES

Station Information		American			Soviet Union		
Incubation Period (hr)	Depth (m)	C-14 DPM$^+$ mean	1 s^{++}	Productivity mg C m^{-3} hr^{-1}	C-14 DPM$^+$ mean	1 s^{++}	Productivity mg C m^{-3} hr^{-1}
#1	0.5	467	53	0.60	357	33	0.46
07/28/75	1.3	515	16	0.66	-	-	-
40 53N	9.5	382	20	0.49	444	51	0.57
71 27W	25.0	501	94	0.64	461	120	0.59
2.0 hr	35.0	559	11	0.72	630	750	0.81
#2	0.5	430	97	0.35	135	100	0.11
07/28/75	1.3	425	10	0.36	258	25	0.22
40 32N	3.5	313	52	0.27	171	102	0.15
71 27W	9.5	229	149	0.20	90	30	0.08
3.0 hr	25.0	208	58	0.18	210	13	0.18
#3	0.5	233	12	0.20	218	24	0.19
07/29/75	1.5	313	27	0.26	809	498	0.68
40 04N	3.5	345	20	0.30	332	58	0.28
71 34W	9.5	210	10	0.18	1214	1763	1.04
3.0 hr	25.0	1096	28	0.95	3239	364	2.81
#4	0.5	315	14	0.26	181	21	0.15
07/29/75	1.3	411	8	0.34	298	20	0.24
34 42N	3.5	335	23	0.27	233	11	0.18
71 54W	9.5	206	11	0.17	161	7	0.13
3.0 hr	25.0	343	49	0.28	357	69	0.29
#7	1	130	5	0.11	147	90	0.13
08/05/75	8	99	21	0.09	113	14	0.10
24 09N	15	1026	126	0.89	115	2	0.10
81 49W	25	44	5	0.04	203	197	0.18
3.0 hr	50	151	7	0.13	166	1	0.14
#8	1	189	51	0.17	121	4	0.11
08/05/75	8	138	42	0.12	113	11	0.10
24 10N	15	129	10	0.11	636	875	0.56
81 50W	25	113	11	0.10	102	8	0.09
3.0 hr	50	84	16	0.08	102	6	0.10
#9*	1	-	-	-	2281	1851	1.94
08/06/75	8	107	69	0.09	202	58	0.17
24 04N	15	26	32	0.02	142	25	0.12
81 57W	25	30	14	0.03	111	23	0.09
3.1 hr	50	103	84	0.09	136	9	0.12
#19**	1	175	43	0.15	74	9	0.06
08/07/75	10	39	3	0.03	57	8	0.05
24 33N	25	49	12	0.04	222	4	0.19
83 45W	50	39	3	0.03	65	11	0.06
3.0 hr	75	43	14	0.06	76	7	0.10

(continued)

TABLE 1 (continued)

Station Information		American C-14 DPM$^+$		Productivity	Soviet Union C-14 DPM$^+$		Productivity
Incubation Period (hr)	Depth (m)	mean	1 s^{++}	mg C m^{-3} hr^{-1}	mean	1 s^{++}	mg C m^{-3} hr^{-1}
#20**	1	51	2	0.05	162	34	0.46
08/08/75	8	35	12	0.03	140	100	0.08
24 31N	15	33	5	0.03	90	5	0.08
83 40W	25	54	18	0.05	71	9	0.06
3.0 hr	50	46	6	0.04	65	2	0.06
#21	1	88	41	0.08	88	30	0.08
08/09/75	8	55	17	0.05	49	23	0.04
24 35N	15	86	9	0.05	44	8	0.04
83 52W	25	79	9	0.07	44	3	0.04
3.2 hr	50	99	55	0.09	52	0	0.05

[+] Carbon-14 activity on filters in disintegrations per minute.

[++] One standard deviation from the sample mean.

[*] Synpor filters used by both sides.

[**] Whatman GF/C filters used by both sides.

Primary productivity values obtained in continental shelf waters off Rhode Island were of the same order as values reported by Ryther and Yentsch (1958). Kabanova and Baluja (1977) obtained primary productivity values in the Straits of Florida similar to those reported here.

Primary productivity values were integrated for each station using linear interpolation between productivity values at discrete sample depths. Integrated productivity values were treated by linear regression (Fig. 1). Station 3 was deleted from the regression due to high variance in Soviet samples. Station 9 was deleted from the regression due to consistent underestimation of productivity by the American method using Synpor filters. Since the large activity value at 15 m, Station 8, obtained by the Soviets was due to a high activity for one sample, that sample was deleted from the treatment. The slope of the regression of American and Soviet productivity values was not significantly different from 1.0 ($p = 0.05$) while the intercept was not significantly different from 0.0 ($p = 0.05$). This suggests that the American and Soviet methods for measuring phytoplankton primary productivity used in this intercalibration gave similar values. Differences in the design of in-situ incubation apparatus and in position of American and Soviet incubation apparatus on the hydrographic wire did not affect the productivity estimates.

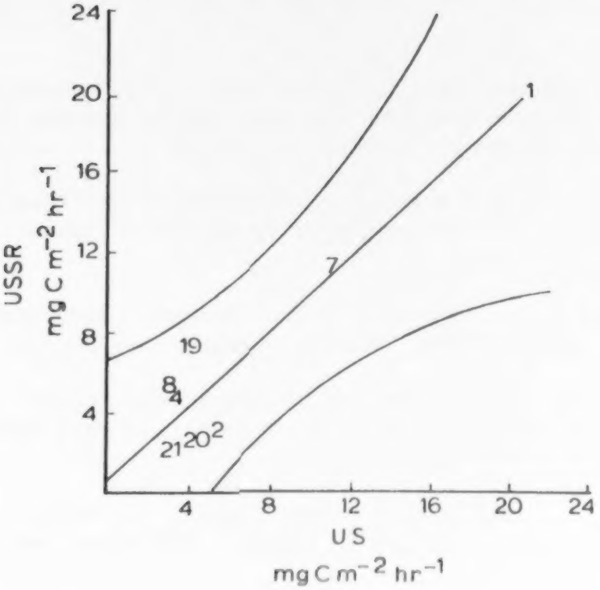

Figure 1. Linear regression of Soviet and American integrated productivity values. The regression equation is $Y = 0.70 + 0.92\ X$ with $r^2 = 0.94$. Numbers indicate stations, 95 percent confidence limits are drawn.

ACKNOWLEDGMENTS

Financial support for this work was provided in the United States by the Environmental Protection Agency and in the Soviet Union by Moscow State University.

Soviet liquid scintillation counting was performed by Drs. Ozrina and Pastuchenko from the Isotope Analysis Laboratory of the Moscow State University Biological Faculty.

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MICROBIOLOGY

Soviet and American investigators have developed "standard" procedures for estimating bacterioplankton uptake. Although a valuable insight into phytoplankton-bacterioplankton relationships, bottle assays are subject to question because they prevent natural diffusion of nutrients and autoinhibitory substances and fail to exclude the effect of predation. A developing methodology based on diffusion cultures using selectively filtered populations as an inoculum and as an estimate of biomass appears worthy of further development. Both methods, together with the analysis of dissolved organic matter during the diurnal cycle to determine gross release and uptake rates, should be useful in investigating the gradation of stress to a specific pollutant in several environments.

It is proposed that an American ship and a Soviet ship visit selected sites to compare the standard ^{14}C bottle assays with the diffusion culture and dissolved organic matter release and uptake for primary and heterotrophic productivity.

Dr. Sieburth expressed the desire that the R/V *Endeavor* be used to study Soviet waters noted for hydrocarbon pollution, while a Soviet vessel conducts similar studies in a comparable U.S. area.

Laboratory studies should be conducted as a supplement and perhaps as a forerunner to the cruises. Personnel from the laboratory of Professor Sieburth, as guests of Professor M. Gusev of the Biological Science Department of MGU in Moscow, would learn his biochemical indicators, which could be used in later research. This group could perhaps visit Yuri I. Sorokin at Galenzhik in the Crimea, at some time in the future. Conversely, Professor Gusev and colleagues would be invited by Professor Sieburth and the Marine Ecosystem Research Laboratory to study energy-related pollution at Narragansett, Rhode Island, in the future.

Arrangements for the laboratory visits should start immediately, with plans for cruises to be formulated in the future.

RADIOACTIVITY

The present levels of radioactive contamination of surface waters of world oceans is about 1000 times lower than the levels of natural radionuclides. In coastal waters and enclosed seas, the concentration of artificial radionuclides is 5-10 times higher than in oceanic water. In fresh water (rivers, lakes, and reservoirs) the concentration of artificial radionuclides is comparable with the concentration of natural radioactive substances. At the same time, it was noted that concentrations of artificial radionuclides have reached significant levels in several marine environments where the discharge of radioactive wastes takes place.

The calculated values of absorbed doses of ionizing radiation which may affect hydrobiota in water bodies of various types presently are lower than background dose rates in the open ocean and approximately equal for freshwater organisms. In some areas where direct discharge of radioactive wastes is taking place, the dose to fish may reach 0.1 rad/day.

Data collected thus far indicate that doses of ionizing radiation in the marine environment have not adversely affected systemic groups, including fish. However, long residence times of fish in a contaminated environment where the dose may approach 0.1 rad/day, may be followed by the lowering of resistance to harmful factors, the destruction of reproductive functions and perhaps a decrease in population size.

As the use of atomic energy increases and as there is increased discharge of radioactive wastes, the area of contamination in the ocean also will grow proportionally. This provides a need to initiate a program of forecast monitoring in regions where radioactive wastes are being discharged. Special attention should be paid to the more potentially dangerous of the long-lived radioisotopes such as plutonium-238, plutonium-239, strontium-90, cesium-137 and several others.

Due to the anticipated growth of nuclear industry, the U.S. National Academy of Sciences has estimated that the world-wide inventory of plutonium will approach 94 x 10^6 Ci by the year 2000. Thus it is imperative that research on migration, accumulation and potential biological effects of plutonium as well as other transuranics and fission products in the marine environment be expanded. In order that this research be conducted most efficiently, we recommend that a joint U.S.-U.S.S.R. workshop on the cycling and biological effects of transuranics and other radionuclides in the marine environment be held. Such a workshop would allow an exchange of views and research information among scientists from both countries.

In addition, perhaps an intercalibration study or cooperative research project could be accomplished prior to the workshop and the data exchanged at that time. If such a recommendation receives favorable consideration, we believe that the workshop should take place in the near future.

ECOSYSTEMS

There is a need to develop procedures for the systematic study of pollutant effects at the ecosystem level. Such studies should include investigations of both unstressed and stressed systems, and must range in objectivity and complexity from simple laboratory microcosms to field ecosystem analysis. At this time, no generally applicable concepts exist for developing such tests under both laboratory and field conditions. Therefore, investigators have no standards for designing such tests or interpreting data derived from them. A standardized methodology is required to provide the ability to compare results from similar systems in different areas and with different pollutants. Microcosms are becoming increasingly recognized as a necessary tool for understanding and finding solutions to both theoretical and practical problems. Therefore, we recommend that the US and USSR continue to emphasize the development of marine and estuarine microcosms that can provide information for water quality management decisions. We further recommend that the US and USSR coordinate their investigations to avoid duplication of effort and enhance the exchange of experience and information. To fulfill this mutual research objective, we propose that:

a. Over the next two years, the US and USSR agree upon and develop common laboratory microcosms (i.e., microbiological; predator-prey, etc.).

b. For the development of these microcosms, provisions should be made for consultation (exchange of information and site visits).

c. To provide a common basis for system design and data interpretation and to develop the common ability to predict environmental impacts, the US and USSR should exchange pollutants (i.e., persistent pesticides), which have global usage. Such pollutants would include those manufactured in each country for distribution throughout the biotic component of the biosphere.

MODELS

Models, viewed as simplified representations of a real-world system, subsystem or function, may be constructed as biological models of the "microcosm-type," words, diagrams, or mathematical equations. As analytical tools, they may be used qualitatively for conceptual and planning purposes or quantitatively for simulation purposes to understand the behavior of the system of interest or for both purposes. Although models merely reflect certain aspects of the system studied and may represent theories or hypotheses, they also have some intrinsic predictive qualities. Formal models, either qualitative or quantitative, become increasingly important scientific tools in posing tractable questions about complex systems, lending emphasis to key factors and eliminating non-essential detail.

A wide variation of opinion seems to exist concerning the number of factors or systems essential to a simulation model (as a system of equations and coefficients) and the degree of investigator control over the operational simulation model (i.e., pre-determined boundary limits vs intrinsic limits established by the model). In contrast, the mathematical techniques are not questioned, although the mathematical approach to modeling may vary (determined models, stochastic models). These opinions range from the inclusion and evaluation of all known components and interactions, to the inclusion and evaluation of only components and interrelationships known or likely to be important in resolving the question(s). Both approaches are considered useful in addressing pollution problems. A distinction was also made between models simulated to solve a problem (an infinite number of constructions) versus those designed to understand the causes of the dynamic behavior of systems (only one best approximation model construction). Regardless, models have a distinct purpose in formulating research goals. The true value of a modeling effort is measured by subsequent verification.

It is proposed that participants in a future US-USSR joint research program use conceptual models and follow through with their parameterization, subsequent simulation, and verification.

American and Soviet specialists should attempt to develop a mathematical model and to provide a quantitative expression of a simple ecosystem. For this purpose, experimental microcosm systems may be used (these could be a pelagic microcosm version or microcosm that includes the substrate and overlying water). Future tasks might include comparison of such models as well as their development and improvement.